N-Cadherin Antibodies Inhibit Growth of Established PC3 Tumors

Treatment started when tumor palpable on Day 13: 10 mg/kg twice weekly for two weeks

FIGURE 17b
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N-CADHERIN: TARGET FOR CANCER DIAGNOSIS AND THERAPY

CROSS-REFERENCES TO RELATED APPLICATIONS


BACKGROUND OF THE INVENTION

[0002] Prostate cancer is the most common malignancy and the second leading cause of cancer-related death in American men. Prostate cancer is a biologically and clinically heterogeneous disease. A majority of men with this malignancy harbor slow-growing tumors that may not impact an individual's natural lifespan, while others are struck by rapidly progressive, metastatic tumors. PSA screening is limited by a lack of specificity and an inability to predict which patients are at risk to develop hormone refractory metastatic disease. Recent studies advocating a lower PSA threshold for diagnosis may increase the number of prostate cancer diagnoses and further complicate the identification of patients with indolent vs. aggressive cancers (Punglia et al, N Engl J Med, 349: 335-342 (2003)). New serum and tissue markers that correlate with clinical outcome or identify patients with potentially aggressive disease are urgently needed (Welsh et al, Proc Natl Acad Sci USA, 100: 3410-3415 (2003)).
Recent expression profiling studies suggest that expression signatures for metastatic vs. non-metastatic tumors may reside in the primary tumor (Ramaswamy et al., Nat Genet, 33: 49-54 (2003); Sotiriou et al, Proc Natl Acad Sci USA, 100: 10393-10398 (2003)). Additional features that predispose tumors to metastasize to specific organs may also be present at some frequency in the primary tumor (Kang et al, Cancer Cell, 3: 537-549 (2003)). These recent observations suggest that novel markers of pre-metastatic or pre-hormone refractory prostate cancer may be identified in early stage disease. These markers may also play a role in the biology of metastatic or hormone refractory prostate cancer progression. Recent examples of genes present in primary tumors that correlate with outcome and play a role in the biology of prostate cancer progression include EZH2 and LIM kinase (Varambally et al., Nature, 419: 624-629 (2002); Yoshioka et al., Proc Natl Acad Sci USA, 100: 7247-7252 (2003)). However, neither of these two genes is secreted.

In order to identify new candidate serum or tissue markers of hormone refractory prostate cancer, we compared gene expression profiles of paired hormone dependent and hormone refractory prostate cancer xenografts. The LAPC-9 xenograft was established from an osteoblastic bone metastasis and progresses from androgen dependence to independence following castration in immune deficient mice (Craft et al, Cancer Research, 59:5030-6 (1999)). It has been used previously to identify candidate therapeutic targets in prostate cancer. Differentially expressed genes were validated and then examined for sequence homology to secreted or cell surface proteins. The identification, characterization and initial validation of N-cadherin, which is expressed in both hormone refractory prostate cancer and bladder cancer, has been previously reported (WO/2007/109347).

We previously disclosed our identification of N-cadherin as a putative diagnostic and therapeutic target in prostate and bladder cancers (WO/2007/109347). Our previous disclosure demonstrated significant expression of the target in high risk and advanced prostate and bladder tumors and showed that expression of the target is associated with poor prognosis and progression to androgen independence. Although there has been previous speculation that N-cadherin might be a useful therapy target, the only existing drug was a peptide antagonist, which did not show any preclinical activity against prostate cancer. To our knowledge, our invention provides the first monoclonal antibodies that are active against cancers expressing the target. In addition, the existing N-cadherin antagonist targets only the first extracellular domain of the protein. We describe antibodies that target the first and fourth extracellular domains. All have significant antitumor activity. To our knowledge, this is the first description of the concept of targeting the fourth extracellular domain. Our antibodies
can be used a single agents, in combination, and also conceptually as agents that can be combined with antagonists of parallel or downstream pathways to N-cadherin.

[0006] The invention encompasses multiple monoclonal antibodies against the first and fourth extracellular domains of the N-cadherin protein. These antibodies block tumor growth, angiogenesis and metastasis in \textit{in vivo} models of prostate and other cancers. They work by blocking N-cadherin signal transduction pathways that are critical for tumor growth, invasion, angiogenesis and metastasis. The antibodies may also be useful for \textit{in vivo} imaging of N-cadherin positive tumors and/or for tissue diagnosis and prognosis.

[0007] The invention can be practiced alone as single antibodies to treat or prevent tumor growth and metastasis. They may be used as adjuvants or as therapeutics for existing tumors. They may be used in combination to block multiple domains of the N-cadherin protein. They may also be used in combination with chemotherapy or other targeted cancer agents, particularly those that target synergistic signal transduction pathways or those that target downstream or upstream pathways involved in N-cadherin mediated signal transduction.

[0008] There are currently no approved therapies or diagnostics targeting N-cadherin. The only drug targeting this pathways has not been highly successful in Phase II trials and has shown no activity in the preclinical models that our invention is active against, suggesting the clear superiority of our approach and our agents.

[0009] Accordingly, the invention provides compositions and methods that target N-cadherin in the diagnosis, prognosis, and treatment of cancers expressing N-cadherin including, but not limited to, prostate cancer and bladder cancer. We also provide a method of treating or diagnosing a cancer patient, wherein the N-cadherin protein in the cancer cells is expressed at normal or low levels, or is expressed by a subset of cancer cells and is not overexpressed. We also report a method of identifying cancer stem cells by determining the presence or absence or amount of the N-cadherin protein in test issue sample.

**BRIEF SUMMARY OF THE INVENTION**

[0010] In one aspect, the present invention provides a hybridoma cell line deposited as ATCC Accession No. PTA-9387 and an antibody produced by this hybridoma cell line, as well as another hybridoma cell line deposited as ATCC Accession No. PTA-9388 and an antibody produced by this hybridoma cell line. Also provided is an antibody or fragment thereof capable of binding to domains 1-3 of N-cadherin, the same antigenic determinant of N-cadherin, \textit{in vivo} or \textit{in vitro}, as does the monoclonal antibody produced by the hybridoma
cell line deposited at the American Type Culture Collection having ATCC Accession No. PTA-9387, or an antibody or fragment thereof capable of binding to domain 4 of N-cadherin, the same antigenic determinant of N-cadherin, \textit{in vivo} or \textit{in vitro}, as does the monoclonal antibody produced by the hybridoma cell line deposited at the American Type Culture Collection having ATCC Accession No. PTA-9388. Such an antibody may be humanized or fully human; or it may be a diabody or single chain antibody (scFv).

[0011] In a second aspect, this invention provides a method of inhibiting the growth of cancer cells in a patient. The method comprises the steps of: administering an antibody (or its fragment) of this invention to a patient under conditions sufficient for binding the antibody (or its fragment) to the cancer cells, which express or overexpress N-cadherin. The antibody (or its fragment) inhibits the growth of cancer cells by (a) activating or inhibiting NF kappa-B signaling and transcription; (b) activating or inhibiting N-cadherin internalization; (c) activating or inhibiting PI3 kinase or Akt pathway; (d) activating or inhibiting \(\beta\)-catenin signaling; (e) blocking heterodimerization of N-cadherin with FGFR or other tyrosine kinase receptor; or (f) blocking or enhancing cleavage by ADAM10 or other metallopeptidase. In some embodiments, the cancer cells are urogenital cancer cells, prostate cancer cells, or bladder cancer cells.

[0012] In a third aspect, the present invention provides a method of treating a cancer patient. The method comprises the steps of: obtaining a test tissue sample from an individual at risk of having a cancer that expresses a N-cadherin protein; determining the presence or absence or amount of the N-cadherin protein in the test tissue sample in comparison to a control tissue sample from an individual known to be negative for the cancer; thereby diagnosing said cancer that expresses a N-cadherin protein, wherein the N-cadherin protein is expressed at normal or low levels, or is expressed by a subset of cells, and wherein the N-cadherin protein is not overexpressed; and administering an effective amount of N-cadherin antibody or fragment thereof to the individual at risk of having a cancer that expresses a N-cadherin protein.

[0013] In some embodiments, the tissue sample is prostate or bladder tissue. In some embodiments, the cancer is a prostate cancer or bladder cancer, or it may be a metastatic cancer. In some embodiments, the antibody (or its fragment) blocks hormone refractory prostate cancer, or antibody blocks cancer stem cells. The antibody in some cases is a monoclonal antibody, an scFv, or a diabody.
[0014] In a fourth aspect, the present invention provides a method of diagnosing a cancer patient. The method comprises the steps of: (a) obtaining a test tissue sample from an individual at risk of having a cancer that expresses a N-cadherin protein; (b) determining the presence or absence or amount of the N-cadherin protein in the test tissue sample in comparison to a control tissue sample from an individual known to be negative for the cancer by contacting a sample with an effective amount of N-cadherin antibody (or its fragment) of this invention; thereby diagnosing said cancer that expresses a N-cadherin protein, wherein the N-cadherin protein is expressed at normal or low levels, or is expressed by a subset of cells, or is overexpressed.

[0015] In a fifth aspect, the present invention provides a method of identifying cancer stem cells. The method comprises the steps of: obtaining a test tissue sample from an individual at risk of having a cancer that expresses a N-cadherin protein; determining the presence or absence of cancer stem cells in the test tissue sample in comparison to a control tissue sample from an individual known to be negative for the cancer; wherein the N-cadherin protein is expressed at normal or low levels, or is expressed by a subset of the stem cells and is not overexpressed. In one embodiment, said tissue sample is prostate or bladder tissue. In another embodiment, said cancer is a prostate cancer. In another embodiment, said cancer is a bladder cancer. In another embodiment, said cancer is a hormone refractory prostate cancer. In another embodiment, said cancer is a metastatic cancer. In another embodiment, said antibody is a monoclonal antibody. In another embodiment, the fragment is a scFv. In another embodiment, the fragment is a diabody.

[0016] In a sixth aspect, the invention provides a method of identifying an anti-N-cadherin antibody or a compound that inhibits the growth of cancer cells in a patient. The method comprises the steps of: (i) contacting the candidate compound or antibody with a cell expressing or overexpressing an N-cadherin polypeptide; and (ii) determining the functional effect of the candidate compound or antibody upon the N-cadherin polypeptide by determining if the compound or antibody: (a) activates or inhibits NF kappa-B signaling and transcription; (b) activates or inhibits N-cadherin internalization; (c) activates or inhibits PI3 kinase or Akt pathway; (d) activates or inhibits β-catenin signaling; (e) blocks heterodimerization of N-cadherin with FGFR or other tyrosine kinase receptor; or (f) blocks or enhances cleavage by ADAM 10 or other metallopeptidase. If the candidate compound or antibody is shown to exhibit activity in either one of (a)-(f), the antibody or compound is deemed an anti-N-cadherin antibody or a compound that inhibits growth of N-cadherin expressing cancers in a patient. For example, the antibody or compound is deemed an anti-
N-cadherin antibody or a compound that inhibits growth of N-cadherin expressing cancers in a patient when the antibody or compound inhibits NF kappa-B signaling or transcription; or when the antibody or compound inhibits N-cadherin internalization.

[0017] In another aspect, the invention provides a preferred epitope for N-cadherin. In some embodiments, the invention provides an antibody that binds to an epitope to which the N-cadherin antibody EC4 binds or competes with the binding of the antibody EC4 to N-cadherin. In another set of embodiments, the epitope has the sequence SDPANWLKIDPVNG (SEQ ID NO: 10). In some embodiments, the antibody for use according to the invention binds to all or any portion of this epitope (including ones that overlap with it). The antibody may be a monoclonal antibody, an scFv fragment, a diabody, or a minibody. In some embodiments, the antibody is a humanized antibody. In some embodiments, the invention provides pharmaceutical compositions comprising said antibody. In other embodiments, a polypeptide comprising the epitope is used to generate antibodies or immune responses against the epitope. Related polypeptide and nucleic acid vaccines directed toward the epitope are also contemplated. The antibodies, polypeptides, and nucleic acids can be used in any aspects of the invention as well, including but not limited to methods of detecting cells expressing N-cadherin in a biological sample, methods of treating, preventing, or ameliorating a disease associated with N-cadherin, methods of identifying a modulator of N-cadherin, or methods of treating a cancer which expresses or overexpresses N-cadherin.

[0018] In another aspect, the invention provides a method of treating a cancer wherein the N-cadherin protein in the cancer cells is expressed at normal or low levels, or is expressed by a subset of cancer cells and is not overexpressed by administering to the patient an antibody according to the invention. We also provide a method of identifying cancer stem cells by determining the presence or absence or amount of the N-cadherin protein in test issue sample.

[0019] In any of the above aspects and embodiments, the tissue, cancer, subject, or patient to be treated is human or mammalian. In any of the above aspects and embodiments, the cancer can be an androgen independent cancer.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[0020] **Figure 1.** Model of N-cadherin signaling in prostate cancer.

[0021] **Figure 2.** N-cadherin activates NF-KB.
[0022] **Figure 3.** N-cadherin activates NF-KB.

[0023] **Figure 4.** N-cadherin is expressed on the cell surface of CI cells.

[0024] **Figure 5.** NF-κB localizes to the nucleus in N-cadherin positive cells (CI).

[0025] **Figure 6.** N-cadherin expression results in induction of IL-6, IL-8, TGFβ2, and bel-2.

[0026] **Figure 7.** Correlation of induced genes with N-cadherin level.

[0027] **Figure 8.** N-cadherin knockdown leads to downregulation of IL-6 and IL-8.

[0028] **Figure 9.** NF-κB activity after N-cadherin antibody treatment.

[0029] **Figure 10.** N-cadherin in PC3 cells: 48-hour antibody incubation.

[0030] **Figure 11.** N-cadherin knockdown leads to downregulation of activated Akt.

[0031] **Figure 12.** N-cadherin specific antibodies activate, then downregulate Akt activation.

[0032] **Figure 13.** FACS analysis of antibody clones targeting the first extracellular domain of the N-cadherin protein.

[0033] **Figure 14.** FACS analysis of antibody clones targeting the fourth extracellular domain of the N-cadherin protein.

[0034] **Figure 15.** FACS analysis of purified monoclonal N-cadherin antibody clones.

[0035] **Figure 16.** *In vitro* invasion assay of monoclonal N-cadherin antibody clones.

[0036] **Figure 17.** (a) Growth curve of N-cadherin null tumors under treatment by antibodies against N-cadherin, 1H7 and EC4, showing no significant inhibitory effect, (b) Tumor growth curve of PC3 prostate cancer cells treated with the same antibodies, demonstrating effectiveness in growth inhibition, (c) Growth curve of large established PC3 tumors under treatment by the 1H7 and EC4 antibodies, (d) Long-term growth curve of PC3 tumors under treatment by the EC4 antibody.

[0037] **Figure 18.** *In vivo* experiment showing effect of antibodies against N-cadherin.

[0038] **Figure 19.** (a) Immunohistochemical staining of an androgen independent LAPC9 prostate cancer, (b) Treatment of androgen dependent and independent LAPC9 tumors with N-cadherin antibodies.
[0039] **Figure 20.** Tumor growth curve of sorted and unsorted LAPC9AI cells.

[0040] **Figures 21 and 22.** FACS results on processed N-cadherin sorted tumors.

[0041] **Figure 23.** Growth curves of LNCaP-Cl tumors, showing inhibitory effects of N-cadherin antibodies.

[0042] **Figure 24.** N-cadherin antibodies inhibit growth of established LAPC-9 androgen independent tumors (a) and large established LAPC-9 androgen independent tumors (b).

[0043] **Figure 25.** Inhibition of PC3 tumor growth in nude mice by antibody EC4 in a dose-correlated manner.

[0044] **Figure 26.** N-cadherin antibodies 1H7 and EC4 show an inhibitory effect on the growth of LAPC-9 androgen dependent tumor in two studies in which the tumor progression was followed for up to 45 days (a) and 70 days (b).

[0045] **Figure 27.** N-cadherin sorted cells show a growth advantage in castrated SCID mice.

[0046] **Figure 28.** Correlation between N-cadherin expression and androgen receptor level in successive passages of LAPC9 tumor cells.

[0047] **Figure 29.** (a) Immunohistochemical staining of an androgen independent LAPC9 prostate cancer, showing that N-cadherin is only expressed by a small subset of cells, (b) Treatment of androgen dependent and independent LAPC-9 tumors with control PBS or N-cadherin antibodies 1H7 and EC4.

[0048] **Figure 30.** N-cadherin positive and negative cells were sorted, yielding a population of cells that were 100% and 0% positive for N-cadherin, respectively.

[0049] **Figures 31 and 32.** FACS analysis of tumors that grew from purely N-cadherin positive and negative cells, vs the control unsorted population.

[0050] **Figure 33.** N-cadherin protein (SEQ ID NO: 1) and mRNA (SEQ ID NO:2) sequences.

[0051] **Figure 34.** N-cadherin variant protein (SEQ ID NO:3) and mRNA (SEQ ID NO:4) sequences and antibody binding information.

[0052] **Figure 35.** Polypeptides (SEQ ID NOs:5-16) used in mapping the N-cadherin epitope bound by EC4.
DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

[0053] We report here on the identification, characterization and validation of a gene product which are expressed in hormone refractory prostate cancer and bladder cancer. These gene product is N-cadherin.

[0054] N-cadherin does not need to be overexpressed compared to normal tissues to be a target for treatment and diagnosis. It can be expressed at low levels and also can be expressed only by a subset of cells. Data shows that targeting N-cadherin even in 5% of prostate cancer cells is sufficient to block the progression of prostate cancers to castration resistance.

[0055] N-cadherin is a target in cancer stem cells. The data showing that targeting of N-cadherin in 5% or fewer cells is sufficient to block tumor progression is consistent with the hypothesis that N-cadherin is a marker of cancer stem cells, and that inhibition of N-cadherin on these stem cells is sufficient to block growth of the tumor as a whole.

[0056] N-cadherin expressing cells, consistent with its being a cancer stem cell marker, are more tumorigenic than N-cadherin non-expressing cells. N-cadherin positive cells can give rise to N-cadherin negative cells, also consistent with the theory that N-cadherin is a novel marker of cancer stem cells. Finally, tumors must upregulate or acquire N-cadherin in order to grow. That is, tumor stem cells must acquire properties of epithelial to mesenchymal transition in order to be tumorigenic.

[0057] Accordingly, N-cadherin is an especially promising therapeutic target for cancer therapy, including but not limited to prostate and bladder cancer. It is found on cell surfaces, overexpressed in many epithelial tumors, and is associated with invasion, metastasis and possibly androgen independence. As shown in the present invention, cancer stem cells show normal or low expression of N-cadherin. Antibodies against N-cadherin therefore are a particularly preferred agent for use in treating cancers, including but not limited to epithelial, urogenital cancers (bladder, prostate), and, more particularly, their invasive or metastasized forms. In some embodiments, monoclonal antibodies directed against an extracellular domain of N-cadherin are preferred. In further embodiments, the first extracellular domain (EC1), portions of the first and second domains, or fourth extracellular domain of N-cadherin are preferred in treating these cancers. In some embodiments, use of a antibody directed toward the extracellular domain 4 is particularly preferred in these treatments as this domain is found to be important in pro-motility and invasive potential (see, Kim et al, J Cell Biol.
incorporated by reference in its entirety with respect to the definition of the various N-cadherin domains.

[0058] The current invention identifies the epitope to which the N-cadherin antibody EC4 binds. The epitope has the sequence SDPANWLKIDPVNG (SEQ ID NO: 10). In some embodiments, the antibody for use according to the invention binds to all or any portion of this epitope (including ones that overlap with it). The epitope is found on the ectodomain of N-cadherin. This invention contemplates any agent (e.g., molecule, antibody, nucleic acid aptamer, peptide, or small molecule) that specifically binds to or inhibits the activity of N-cadherin by binding to the epitope or a portion of the epitope and the use of such in a method according to the invention. This particular epitope mediates multiple properties of tumor growth and to which an antibody can bind and inhibit these properties to produce diverse antitumor or signaling effects.

[0059] Accordingly, in some embodiments, the invention provides an N-cadherin antibody that binds to the N-cadherin epitope SDPANWLKIDPVNG (SEQ ID NO: 10) or any portion thereof. The antibody may be a monoclonal antibody, a diabody, a minibody, a triabody. The antibody may be linked to a therapeutic agent, a diagnostic agent, or a detectable label. The antibody may be a humanized antibody. In some embodiments, the antibody competes with EC4 for binding to N-cadherin or binds N-cadherin within the sequence SDPANWLKIDPVNGQITTIAVL (SEQ ID NO: 17) or QQNIRYTKLSDPANWLKIDPVNGQITTIAVL (SEQ ID NO: 18).

[0060] In some embodiments, the invention provides pharmaceutical compositions comprising a pharmaceutically acceptable excipient and the above antibodies directed toward the SDPANWLKIDPVNG portion of N-cadherin. In some embodiments, the antibody of the pharmaceutical composition is a monclonal antibody, an scFv fragment, a diabody, a minibody, or a triabody. In some embodiments, the antibody of the pharmaceutical composition is a humanized antibody. In some embodiments, the antibody of the pharmaceutical composition competes with EC4 for binding to N-cadherin or binds N-cadherin within the sequence SDPANWLKIDPVNGQITTIAVL (SEQ ID NO: 17) or QQNIRYTKLSDPANWLKIDPVNGQITTIAVL (SEQ ID NO: 18). In some embodiments, the antibody of the pharmaceutical composition is linked to a therapeutic agent, a diagnostic agent, or a detectable label.

[0061] In still other embodiments, the invention provides methods of treating, preventing or ameliorating a disease associated with the overexpression of N-cadherin by administering an
N-cadherin antibody that binds to the N-cadherin epitope SDPANWKDPVNG (SEQ ID NO: 10) or any portion thereof to an individual in need thereof. In some embodiments, the antibody that is administered is a monoclonal antibody, an scFv fragment, a diabody, a minibody, or a triabody. In some embodiments, the antibody that is administered is a humanized antibody. In some embodiments, the antibody that is administered competes with EC4 for binding to N-cadherin or binds N-cadherin within the sequence SDPANWKDPVNGQITTIAVL (SEQ ID NO: 17) or QQNIRYTKLSDPANWKDPVNGQITTIAVL (SEQ ID NO: 18). In some embodiments, the antibody that is administered is linked to a therapeutic agent, a diagnostic agent, or a detectable label. In some further embodiments, the proliferation or metastasis of a cancer cell in the individual is inhibited following the administering step.

[0062] In still other embodiments, the invention provides a method of treating a cancer, including but not limited to, for instance, bladder cancer and prostate cancer, associated with tumor cells that express, or overexpress an N-cadherin protein by administering an N-cadherin antibody that binds to the N-cadherin epitope SDPANWKDPVNG (SEQ ID NO: 10) or any portion thereof to an individual in need thereof. In some embodiments, the antibody that is administered is a monoclonal antibody, an scFv fragment, a diabody, a minibody, or a triabody. In some embodiments, the antibody that is administered is a humanized antibody. In some embodiments, the antibody that is administered competes with EC4 for binding to N-cadherin or binds N-cadherin within the sequence SDPANWKDPVNGQITTIAVL (SEQ ID NO: 17) or QQNIRYTKLSDPANWKDPVNGQITTIAVL (SEQ ID NO: 18). In some embodiments, the antibody that is administered is linked to a therapeutic agent, a diagnostic agent, or a detectable label.

[0063] In other embodiments, the invention provides methods of detecting a cell expressing N-cadherin in a biological sample, the method comprising contacting the biological sample with an antibody directed to the SDPANWKDPVNG portion of N-cadherin and detecting the presence of the antibody. In still other embodiments, the invention provides methods for identifying a modulator of N-cadherin comprising (a) contacting a test agent to a polypeptide of up to 15, 20, 25, 30 or 35 amino acids in length and comprising the amino acid sequence SDPANWKDPVNG (SEQ ID NO: 10); and (b) selecting an agent that binds to the polypeptide, wherein binding of the test agent to the polypeptide is an indication that the test agent is a modulator of N-cadherin activity. In still further embodiments, the selecting step
comprises measuring the ability of the test agent to compete with EC4 for binding to the polypeptide.

[0064] In some embodiments, the invention provides a compound which is a polypeptide for use according to the invention consisting of at least 24 contiguous amino acids of the sequence QQNIRYTKLSDPANWLKIDPVNGQITTIAVLD (SEQ ID NO: 18). In other embodiments, the invention provides a polypeptide consisting at least 14, 15, 16, 17, 20, 25 or 30 contiguous amino acids of N-cadherin and including the sequence SDPANWLKIDPVNG (SEQ ID NO: 10). In other embodiments, the invention provides a polypeptide having the sequence SDPANWLKIDPVNG (SEQ ID NO: 10).

[0065] In other embodiments, the invention provides nucleic acids and vectors encoding such polypeptides. In some embodiments, the nucleic acid or vector comprising the nucleic acid encodes a polypeptide consisting of at least 24 contiguous amino acids of the sequence QQNIRYTKLSDPANWLKIDPVNGQITTIAVLD (SEQ ID NO: 18). In some embodiments, the nucleic acid or vector comprising the nucleic acid encodes a polypeptide consisting at least 14, 15, 16, 17, 20, 25 or 30 contiguous amino acids of N-cadherin and including the sequence SDPANWLKIDPVNG (SEQ ID NO: 10). In some embodiments, the nucleic acid or vector comprising the nucleic acid encodes a polypeptide having the sequence SDPANWLKIDPVNG (SEQ ID NO: 10).

[0066] In still other embodiments, the polypeptides, nucleic acids, or vectors are formulated for administration as vaccines or in the production of antibodies for use according to the invention. In some embodiments, the vaccine comprises a polypeptide consisting of at least 24 contiguous amino acids of the sequence QQNIRYTKLSDPANWLKIDPVNGQITTIAVLD (SEQ ID NO: 18). In some embodiments, the vaccine comprises a polypeptide consisting at least 14, 15, 16, 17, 20, 25 or 30 contiguous amino acids of N-cadherin and including the sequence SDPANWLKIDPVNG (SEQ ID NO: 10). In some embodiments, the vaccine comprises a polypeptide having the sequence SDPANWLKIDPVNG (SEQ ID NO: 10).

[0067] The antibodies and anti-N-cadherin compounds according to the invention have global antitumor properties in vitro or in vivo, especially inhibition of proliferation, causation of apoptosis, inhibition of angiogenesis, reversion of epithelial to mesenchymal transition, castration resistance (in the case of prostate cancer), or in vivo invasion and metastasis.

[0068] N-cadherin expression can contribute to prostate and bladder cancer invasion and metastasis as well as the progression of prostate cancer to hormone refractory disease. N-
cadherin can be targeted therapeutically both alone and in combination with other small molecule inhibitors of mTOR and EGFR. Targeting N-cadherin can help prevent or control invasive and metastatic prostate cancer.

[0069] The present invention discloses two mouse hybridoma cell lines that produces monoclonal antibody capable of binding to the antigenic determinant of N-cadherin. The mouse hybridoma cell lines are deposited as ATCC Accession No. PTA-9387 (1H7) and ATCC Accession No. PTA-9388 (EC4).

[0070] In one embodiment of the invention, an antibody is produced by the hybridoma cell line designated as ATCC Accession No. PTA-9387. In another embodiment of the invention, an antibody is produced by the hybridoma cell line designated as ATCC Accession No. PTA-9388.

[0071] The cells were deposited as ATCC Accession No. PTA-9387 and ATCC Accession No. PTA-9388 pursuant to the Budapest Treaty at the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110, on July 23, 2008. Viability was tested and certified on August 29, 2008.

[0072] In some embodiments of the invention, an antibody or fragment thereof capable of binding to the same antigenic determinant of N-cadherin, *in vivo* or *in vitro*, as does the monoclonal antibody produced by the hybridoma cell line deposited at the American Type Culture Collection having ATCC Accession No. PTA-9387 is produced. In some embodiments of the invention, an antibody or fragment thereof capable of binding to the same antigenic determinant of N-cadherin, *in vivo* or *in vitro*, as does the monoclonal antibody produced by the hybridoma cell line deposited at the American Type Culture Collection having ATCC Accession No. PTA-9388 is produced.

[0073] In some embodiments, the antibody is capable of binding to the first extracellular domain of N-cadherin. In some embodiments, the antibody is capable of binding to the second extracellular domain of N-cadherin. In some embodiments, the antibody is capable of binding to the third extracellular domain of N-cadherin. In some embodiments, the antibody is capable of binding to the first to third extracellular domains of N-cadherin. In some embodiments, the antibody is capable of binding to the fourth extracellular domain of N-cadherin.

[0074] The present invention further relates to methods of inhibiting the growth, or killing, of cancer cells in a patient. The methods generally comprise administering the antibody or
binding fragment thereof to a patient under conditions sufficient for binding the antibody or binding fragment thereof to said tumor cells or prostate cancer tumor cells; modulating cellular activity; inhibiting angiogenesis of the tumor cells; and causing growth inhibition or killing of the tumor cells, wherein the cancer cells express or overexpress N-Cadherin.

[0075] In some embodiments of the invention, the antibody modulates cellular activity by activating or inhibiting NF kappa-B signaling and transcription. In some embodiments of the invention, the antibody modulates cellular activity by activating or inhibiting N-cadherin internalization. In some embodiments of the invention, the antibody modulates cellular activity by activating or inhibiting PI3 kinase or Akt pathway. In some embodiments of the invention, the antibody modulates cellular activity by activating or inhibiting β-catenin signaling. In some embodiments of the invention, the antibody modulates cellular activity by blocking heterodimerization of N-cadherin with FGFR or other tyrosine kinase receptor. In some other embodiments of the invention, the antibody modulates cellular activity by blocking or enhancing cleavage by ADAM 10 or other metalloproteinase.

[0076] In some embodiments, the antibody modulates cellular activity by inhibiting the growth of, or killing, urogenital cancer cells in a patient. In some embodiments, the antibody modulates cellular activity by inhibiting the growth of, or killing, prostate cancer cells in a patient. In some other embodiments, the antibody modulates cellular activity by inhibiting the growth of, or killing, bladder cancer cells in a patient.

II. Definitions

[0077] "N-cadherin" refers to nucleic acids, e.g., gene, pre-mRNA, mRNA, and polypeptides, polymorphic variants, alleles, mutants, and interspecies homologs that: (1) have an amino acid sequence that has greater than about 60% amino acid sequence identity, 65%, 70%, 75%, 80%, 85%, 90%, preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or greater amino acid sequence identity, preferably over a region of over a region of at least about 25, 50, 100, 200, 500, 1000, or more amino acids, to a polypeptide encoded by a respectively referenced nucleic acid or an amino acid sequence described herein, for example, as depicted in GenBank Accession Nos. NM_001792 (N-Cadherin mRNA) and NP_001783 (N-Cadherin protein); (2) specifically bind to antibodies, e.g., polyclonal antibodies, raised against an immunogen comprising a referenced amino acid sequence as depicted in GenBank Accession No. NP_001783 (N-Cadherin protein); immunogenic fragments respectively thereof, and conservatively modified variants respectively thereof; (3) specifically hybridize
under stringent hybridization conditions to a nucleic acid encoding a referenced amino acid sequence as depicted in GenBank Accession No. NP_001783 (N-Cadherin protein) and conservatively modified variants respectively thereof; (4) have a nucleic acid sequence that has greater than about 95%, preferably greater than about 96%, 97%, 98%, 99%, or higher nucleotide sequence identity, preferably over a region of at least about 25, 50, 100, 150, 200, 250, 500, 1000, or more nucleotides, to a reference nucleic acid sequence as shown in GenBank Accession No. NM_001792 (N-Cadherin mRNA). A polynucleotide or polypeptide sequence is typically from a mammal including, but not limited to, primate, e.g., human; rodent, e.g., rat, mouse, hamster; cow, pig, horse, sheep, or any mammal. The nucleic acids and proteins of the invention include both naturally occurring or recombinant molecules.

[0078] "Cancer" refers to human cancers and carcinomas, sarcomas, adenocarcinomas, lymphomas, leukemias, etc., including solid tumors and lymphoid cancers, kidney, breast, lung, kidney, bladder, colon, ovarian, prostate, pancreas, stomach, brain, head and neck, skin, uterine, testicular, esophagus, and liver cancer, lymphoma, including non-Hodgkin's and Hodgkin's lymphoma, leukemia, and multiple myeloma. "Urogenital cancer" refers to human cancers of urinary tract and genital tissues, including but not limited to kidney, bladder, urinary tract, urethra, prostate, penis, testicle, vulva, vagina, cervical and ovary tissues.

[0079] The cancer to be treated herein may be one characterized by low or normal expression (but not overexpression) of N-cadherin. In one embodiment of the invention, a diagnostic or prognostic assay will be performed to determine whether the patient's cancer is characterized by low or normal expression of N-cadherin. Various assays for determining such expression are contemplated and include the immunohistochemistry, FISH and shed antigen assays, southern blotting, or PCR techniques. Moreover, the N-cadherin expression or amplification may be evaluated using an in vivo diagnostic assay, e.g. by administering a molecule (such as an antibody) which binds the molecule to be detected and is tagged with a detectable label (e.g. a radioactive isotope) and externally scanning the patient for localization of the label. In some embodiments, the cancer or cancer stem cell to be treated is not yet invasive, but expresses N-cadherin.

[0080] "Therapy resistant" cancers, tumor cells, and tumors refers to cancers that have become resistant or refractory to either or both apoptosis-mediated (e.g., through death receptor cell signaling, for example, Fas ligand receptor, TRAIL receptors, TNF-R1, chemotherapeutic drugs, radiation) and non-apoptosis mediated (e.g., toxic drugs, chemicals)
cancer therapies, including chemotherapy, hormonal therapy, radiotherapy, and immunotherapy.

[0081] "Low or normal expression" refers to RNA or protein expression of N-cadherin in a test tissue sample that is about the same or lower than RNA or protein expression of N-cadherin in a control tissue sample. In one embodiment, the tissue sample is autologous.

[0082] "Overexpression" refers to RNA or protein expression of N-cadherin in a test tissue sample that is significantly higher that RNA or protein expression of N-cadherin in a control tissue sample. In one embodiment, the tissue sample is autologous. Cancerous test tissue samples (e.g., bladder, prostate) associated with invasiveness, metastasis, hormone independent (e.g., androgen independence), or refractoriness to treatment or an increased likelihood of same typically have at least two fold higher expression of N-cadherin mRNA or protein, often up to three, four, five, eight, ten or more fold higher expression of N-cadherin, in comparison to cancer tissues from patients who are less likely to progress to metastasis or to normal (i.e., non-cancer) tissue samples. Such differences may be readily apparent when viewing the bands of gels with approximately similarly loaded with test and controls samples. Prostate cancers expressing increased amounts of N-cadherin are more likely to become invasive, metastasize, or progress to androgen independent or treatment refractory cancer. Various cutoffs are pertinent for N-cadherin positivity, since it is possible that a small percentage of N-cadherin positive cells in primary tumors may identify tumors with a high risk for recurrence and metastasis. The terms "overexpress," "overexpression" or "overexpressed" interchangeably refer to a gene that is transcribed or translated at a detectably greater level, usually in a cancer cell, in comparison to a normal cell. Overexpression therefore refers to both overexpression of protein and RNA (due to increased transcription, post transcriptional processing, translation, post translational processing, altered stability, and altered protein degradation), as well as local overexpression due to altered protein traffic patterns (increased nuclear localization), and augmented functional activity, e.g., as in an increased enzyme hydrolysis of substrate. Overexpression can also be by 50%, 60%, 70%, 80%, 90%, or more in comparison to a normal cell or comparison cell (e.g., a benign prostatic hyperplasia (BPH) cell).

[0083] The terms "cancer that expresses N-cadherin" and "cancer associated with the expression of N-cadherin" interchangeably refer to cancer cells or tissues that express N-cadherin in accordance with the above definition.
The terms "cancer-associated antigen" or "tumor-specific marker" or "tumor marker" interchangeably refers to a molecule (typically protein, carbohydrate or lipid) that is preferentially expressed in a cancer cell in comparison to a normal cell, and which is useful for the preferential targeting of a pharmacological agent to the cancer cell. A marker or antigen can be expressed on the cell surface or intracellularly. Oftentimes, a cancer-associated antigen is a molecule that is overexpressed or stabilized with minimal degradation in a cancer cell in comparison to a normal cell, for instance, 2-fold overexpression, 3-fold overexpression or more in comparison to a normal cell. Oftentimes, a cancer-associated antigen is a molecule that is inappropriately synthesized in the cancer cell, for instance, a molecule that contains deletions, additions or mutations in comparison to the molecule expressed on a normal cell. Oftentimes, a cancer-associated antigen will be expressed exclusively in a cancer cell and not synthesized or expressed in a normal cell. Exemplified cell surface tumor markers include the proteins c-erbB-2 and human epidermal growth factor receptor (HER) for breast cancer, PSMA for prostate cancer, and carbohydrate mucins in numerous cancers, including breast, ovarian and colorectal. Exemplified intracellular tumor markers include, for example, mutated tumor suppressor or cell cycle proteins, including p53.

An "agonist" refers to an agent that binds to a polypeptide or polynucleotide of the invention, stimulates, increases, activates, facilitates, enhances activation, sensitizes or up regulates the activity or expression of a polypeptide or polynucleotide of the invention.

An "antagonist" refers to an agent that inhibits expression of a polypeptide or polynucleotide of the invention or binds to, partially or totally blocks stimulation, decreases, prevents, delays activation, inactivates, desensitizes, or down regulates the activity of a polypeptide or polynucleotide of the invention.

"Inhibitors," "activators," and "modulators" of expression or of activity are used to refer to inhibitory, activating, or modulating molecules, respectively, identified using in vitro and in vivo assays for expression or activity, e.g., ligands, agonists, antagonists, and their homologs and mimetics. The term "modulator" includes inhibitors and activators. Inhibitors are agents that, e.g., inhibit expression of a polypeptide or polynucleotide of the invention or bind to, partially or totally block stimulation or enzymatic activity, decrease, prevent, delay activation, inactivate, desensitize, or down regulate the activity of a polypeptide or polynucleotide of the invention, e.g., antagonists. Activators are agents that, e.g., induce or activate the expression of a polypeptide or polynucleotide of the invention or bind to, stimulate, increase, open, activate, facilitate, enhance activation or enzymatic activity, sensitize or up regulate the activity of a polypeptide or polynucleotide of the invention, e.g.,
agonists. Modulators include naturally occurring and synthetic ligands, antagonists, agonists, small chemical molecules and the like. Assays to identify inhibitors and activators include, e.g., applying putative modulator compounds to cells, in the presence or absence of a polypeptide or polynucleotide of the invention and then determining the functional effects on a polypeptide or polynucleotide of the invention activity. Samples or assays comprising a polypeptide or polynucleotide of the invention that are treated with a potential activator, inhibitor, or modulator are compared to control samples without the inhibitor, activator, or modulator to examine the extent of effect. Control samples (untreated with modulators) are assigned a relative activity value of 100%. Inhibition is achieved when the activity value of a polypeptide or polynucleotide of the invention relative to the control is about 80%, optionally 50% or 25-1%. Activation is achieved when the activity value of a polypeptide or polynucleotide of the invention relative to the control is 110%, optionally 150%, optionally 200-500%, or 1000-3000% higher.

[0088] The term "test compound" or "drug candidate" or "modulator" or grammatical equivalents as used herein describes any molecule, either naturally occurring or synthetic, e.g., protein, oligopeptide (e.g., from about 5 to about 25 amino acids in length, preferably from about 10 to 20 or 12 to 18 amino acids in length, preferably 12, 15, or 18 amino acids in length), small organic molecule, polysaccharide, lipid, fatty acid, polynucleotide, RNAi, siRNA, antibody, oligonucleotide, etc. The test compound can be in the form of a library of test compounds, such as a combinatorial or randomized library that provides a sufficient range of diversity. Test compounds are optionally linked to a fusion partner, e.g., targeting compounds, rescue compounds, dimerization compounds, stabilizing compounds, addressable compounds, and other functional moieties. Conventionally, new chemical entities with useful properties are generated by identifying a test compound (called a "lead compound") with some desirable property or activity, e.g., inhibiting activity, creating variants of the lead compound, and evaluating the property and activity of those variant compounds. Often, high throughput screening (HTS) methods are employed for such an analysis.

[0089] A "small organic molecule" refers to an organic molecule, either naturally occurring or synthetic, that has a molecular weight of more than about 50 Daltons and less than about 2500 Daltons, preferably less than about 2000 Daltons, preferably between about 100 to about 1000 Daltons, more preferably between about 200 to about 500 Daltons.

[0090] Cytotoxic agents include "cell-cycle-specific" or "antimitotic" or "cytoskeletal-interacting" drugs. These terms interchangeably refer to any pharmacological agent that
blocks cells in mitosis. Such agents are useful in chemotherapy. Generally, cell-cycle-specific drugs bind to the cytoskeletal protein tubulin and block the ability of tubulin to polymerize into microtubules, resulting in the arrest of cell division at metaphase. Exemplified cell-cycle-specific drugs include vinca alkaloids, taxanes, colchicine, and podophyllotoxin. Exemplified vinca alkaloids include vinblastine, vincristine, vindesine and vinorelbine. Exemplified taxanes include paclitaxel and docetaxel. Another example of a cytoskeletal-interacting drug includes 2-methoxyestradiol.

[0091] Construction of suitable vectors containing the desired therapeutic gene coding and control sequences employs standard ligation and restriction techniques, which are well understood in the art (see Maniatis et al., in Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1982)). Isolated plasmids, DNA sequences, or synthesized oligonucleotides are cleaved, tailored, and re-ligated in the form desired.

[0092] Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are near each other, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[0093] "Determining the functional effect" refers to assaying for a compound that increases or decreases a parameter that is indirectly or directly under the influence of a polynucleotide or polypeptide of the invention, e.g., measuring physical and chemical or phenotypic effects. Such functional effects can be measured by any means known to those skilled in the art, e.g., changes in spectroscopic (e.g., fluorescence, absorbance, refractive index), hydrodynamic (e.g., shape), chromatographic, or solubility properties for the protein; measuring inducible markers or transcriptional activation of the protein; measuring binding activity or binding assays, e.g. binding to antibodies; measuring changes in ligand binding affinity; measurement of calcium influx; measurement of the accumulation of an enzymatic product of a polypeptide of the invention or depletion of an substrate; changes in enzymatic activity, e.g., kinase activity, measurement of changes in protein levels of a polypeptide of
the invention; measurement of RNA stability; G-protein binding; GPCR phosphorylation or
dephosphorylation; signal transduction, e.g., receptor-ligand interactions, second messenger
concentrations (e.g., cAMP, IP3, or intracellular Ca2+); identification of downstream or
reporter gene expression (CAT, luciferase, β-gal, GFP and the like), e.g., via
chemiluminescence, fluorescence, colorimetric reactions, antibody binding, inducible
markers, and ligand binding assays.

[0094] Samples or assays comprising a nucleic acid or protein disclosed herein that are
treated with a potential activator, inhibitor, or modulator are compared to control samples
without the inhibitor, activator, or modulator to examine the extent of inhibition. Control
samples (untreated with inhibitors) are assigned a relative protein activity value of 100%.
Inhibition is achieved when the activity value relative to the control is about 80%, preferably
50%, more preferably 25-0%. Activation is achieved when the activity value relative to the
control (untreated with activators) is 110%, more preferably 150%, more preferably 200-
500%, (i.e., two to five fold higher relative to the control), more preferably 1000-3000%,
higher.

[0095] "Biological sample" includes sections of tissues such as biopsy and autopsy
samples, and frozen sections taken for histological purposes. Such samples include blood
and blood fractions or products (e.g., serum, plasma, platelets, red blood cells, and the like),
sputum, tissue, cultured cells, e.g., primary cultures, explants, and transformed cells, stool,
urine, etc. A biological sample is typically obtained from a eukaryotic organism, most
preferably a mammal such as a primate e.g., chimpanzee or human; cow; dog; cat; a rodent,
e.g., guinea pig, rat, Mouse; rabbit; or a bird; reptile; or fish.

[0096] A "biopsy" refers to the process of removing a tissue sample for diagnostic or
prognostic evaluation, and to the tissue specimen itself. Any biopsy technique known in the
art can be applied to the diagnostic and prognostic methods of the present invention. The
biopsy technique applied will depend on the tissue type to be evaluated (i.e., prostate, lymph
node, liver, bone marrow, blood cell), the size and type of the tumor (i.e., solid or suspended
(i.e., blood or ascites)), among other factors. Representative biopsy techniques include
excisional biopsy, incisional biopsy, needle biopsy, surgical biopsy, and bone marrow biopsy.
An "excisional biopsy" refers to the removal of an entire tumor mass with a small margin of
normal tissue surrounding it. An "incisional biopsy" refers to the removal of a wedge of
tissue that includes a cross-sectional diameter of the tumor. A diagnosis or prognosis made
by endoscopy or fluoroscopy can require a "core-needle biopsy" of the tumor mass, or a
"fine-needle aspiration biopsy" which generally obtains a suspension of cells from within the
tumor mass. Biopsy techniques are discussed, for example, in Harrison's Principles of Internal Medicine, Kasper, et al., eds., 16th ed., 2005, Chapter 70, and throughout Part V.

[0097] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides that are the same, as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection. See e.g., the NCBI web site at ncbi.nlm.nih.gov/BLAST. Two sequences that are the same (i.e., have 100% identity) are said to be "identical." Two sequences that have a specified percentage of nucleotides that are the same (e.g., at least about 70% identity, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity over a specified region, when compared and aligned for maximum correspondence over a comparison window or designated region) are said to be "substantially identical." This definition also refers to, or may be applied to, the complement of a test sequence. The definition also includes sequences that have deletions and/or additions, as well as those that have substitutions. As described below, the preferred algorithms can account for gaps and the like. Preferably, identity exists over a region that is at least about 25 amino acids or nucleotides in length, or more preferably over a region that is 50-100 amino acids or nucleotides in length.

[0098] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Preferably, default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0099] A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA
85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (see, e.g., Current Protocols in Molecular Biology (Ausubel et al., eds. 1995 supplement)).

[0100] A preferred example of algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al, Nuc. Acids Res. 25:3389-3402 (1977) and Altschul et al, J. Mol. Biol. 215:403-410 (1990), respectively. BLAST and BLAST 2.0 are used, with the parameters described herein, to determine percent sequence identity for the nucleic acids and proteins of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al, supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

[0101] "Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form, and complements thereof. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone
residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzner et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

A particular nucleic acid sequence also implicitly encompasses "splice variants." Similarly, a particular protein encoded by a nucleic acid implicitly encompasses any protein encoded by a splice variant of that nucleic acid. "Splice variants," as the name suggests, are products of alternative splicing of a gene. After transcription, an initial nucleic acid transcript may be spliced such that different (alternate) nucleic acid splice products encode different polypeptides. Mechanisms for the production of splice variants vary, but include alternate splicing of exons. Alternate polypeptides derived from the same nucleic acid by read-through transcription are also encompassed by this definition. Any products of a splicing reaction, including recombinant forms of the splice products, are included in this definition. An example of potassium channel splice variants is discussed in Leicher, et al., J. Biol. Chem. 273(52):35095-35101 (1998).

The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the
genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an a carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0106] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0107] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence with respect to the expression product, but not with respect to actual probe sequences.

[0108] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution
of an amino acid with a chemically similar amino acid. Conservative substitution tables
providing functionally similar amino acids are well known in the art. Such conservatively
modified variants are in addition to and do not exclude polymorphic variants, interspecies
homologs, and alleles of the invention.

[0109] The following eight groups each contain amino acids that are conservative
substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic
acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I),
Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan
(W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton,
Proteins (1984)).

[0110] A "label" or a "detectable moiety" is a composition detectable by spectroscopic,
photochemical, biochemical, immunochemical, chemical, or other physical means. For
example, useful labels include $^{32}$P, fluorescent dyes, electron-dense reagents, enzymes (e.g.,
as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins which can be
made detectable, e.g., by incorporating a radiolabel into the peptide or used to detect
antibodies specifically reactive with the peptide.

[0111] The term "recombinant" when used with reference, e.g., to a cell, or nucleic acid,
protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by
the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic
acid or protein, or that the cell is derived from a cell so modified. Thus, for example,
recombinant cells express genes that are not found within the native (non-recombinant) form
of the cell or express native genes that are otherwise abnormally expressed, under expressed
or not expressed at all.

[0112] The term "heterologous" when used with reference to portions of a nucleic acid
indicates that the nucleic acid comprises two or more subsequences that are not found in the
same relationship to each other in nature. For instance, the nucleic acid is typically
recombinantly produced, having two or more sequences from unrelated genes arranged to
make a new functional nucleic acid, e.g., a promoter from one source and a coding region
from another source. Similarly, a heterologous protein indicates that the protein comprises
two or more subsequences that are not found in the same relationship to each other in nature
(e.g., a fusion protein).

[0113] The phrase "stringent hybridization conditions" refers to conditions under which a
probe will hybridize to its target subsequence, typically in a complex mixture of nucleic
acids, but to no other sequences. Stringent conditions are sequence-dependent and will be
different in different circumstances. Longer sequences hybridize specifically at higher
temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen,
Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Probes,
"Overview of principles of hybridization and the strategy of nucleic acid assays" (1993).
Generally, stringent conditions are selected to be about 5-10°C lower than the thermal
melting point (Tm) for the specific sequence at a defined ionic strength pH. The Tm is the
temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of
the probes complementary to the target hybridize to the target sequence at equilibrium (as
the target sequences are present in excess, at Tm' 50% of the probes are occupied at equilibrium).
Stringent conditions may also be achieved with the addition of destabilizing agents such as
formamide. For selective or specific hybridization, a positive signal is at least two times
background, preferably 10 times background hybridization. Exemplary stringent
hybridization conditions can be as following: 50%> formamide, 5x SSC, and 1%> SDS,
incubating at 42°C, or, 5x SSC, 1% SDS, incubating at 65°C, with wash in 0.2x SSC, and
0.1% SDS at 65°C.

[0114] Nucleic acids that do not hybridize to each other under stringent conditions are still
substantially identical if the polypeptides which they encode are substantially identical. This
occurs, for example, when a copy of a nucleic acid is created using the maximum codon
degeneracy permitted by the genetic code. In such cases, the nucleic acids typically hybridize
under moderately stringent hybridization conditions. Exemplary "moderately stringent
hybridization conditions" include a hybridization in a buffer of 40% formamide, 1 M NaCl,
1% SDS at 37°C, and a wash in IX SSC at 45°C. A positive hybridization is at least twice
background. Those of ordinary skill will readily recognize that alternative hybridization and
wash conditions can be utilized to provide conditions of similar stringency. Additional
guidelines for determining hybridization parameters are provided in numerous reference, e.g.,

[0115] For PCR, a temperature of about 36°C is typical for low stringency amplification,
although annealing temperatures may vary between about 32°C and 48°C depending on
primer length. For high stringency PCR amplification, a temperature of about 62°C is
typical, although high stringency annealing temperatures can range from about 50°C to about
65°C, depending on the primer length and specificity. Typical cycle conditions for both high
and low stringency amplifications include a denaturation phase of 90°C - 95°C for 30 sec - 2
min., an annealing phase lasting 30 sec. - 2 min., and an extension phase of about 72°C for 1

[01 16] "Antibody" refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. Typically, the antigen-binding region of an antibody will be most critical in specificity and affinity of binding.

[01 17] An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

[01 18] Antibodies exist, e.g., as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab)′_2, a dimer of Fab which itself is a light chain joined to V_H-C_H by a disulfide bond. The F(ab)′_2 may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the F(ab)′_2 dimer into an Fab′ monomer. The Fab′ monomer is essentially Fab with part of the hinge region (see Fundamental Immunology (Paul ed., 3d ed. 1993). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized de novo either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized de novo using recombinant DNA methodologies (e.g., single chain Fv) or those identified using phage display libraries (see, e.g., McCafferty et al., Nature 348:552-554 (1990)). See, also, U.S. Patent Application Serial No. 12/590,601, which is assigned to the same assignee as the present application, and which is incorporated herein by reference in its entirety, and also, particularly with respect to phage display library methods as applied to N-cadherin and the N-cadherin antibodies disclosed therein.
Accordingly, the term antibody also embraces minibodies, diabodies, triabodies and the like. Diabodies are small bivalent bispecific antibody fragments with high avidity and specificity. Their high signal to noise ratio is typically better due to a better specificity and fast blood clearance increasing their potential for diagnostic and therapeutic targeting of specific antigen (Sundaresan et al., J Nucl Med 44:1962-9 (2003). In addition, these antibodies are advantageous because they can be engineered if necessary as different types of antibody fragments ranging from a small single chain Fv to an intact IgG with varying isoforms (Wu & Senter, Nat. Biotechnol. 23:1 137-1 146 (2005)). In some embodiments, the antibody fragment is part of a diabody.

Diabodies, first described by Hollinger et al, PNAS (USA) 90(14): 6444-6448 (1993), may be constructed using heavy and light chains disclosed herein, as well as by using individual CDR regions disclosed herein. Typically, diabody fragments comprise a heavy chain variable domain (V\textsubscript{H}) connected to a light chain variable domain (V\textsubscript{L}) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V\textsubscript{H} and V\textsubscript{L} domains of one fragment are forced to pair with the complementary V\textsubscript{H} and V\textsubscript{L} domains of another fragment, thereby forming two antigen-binding sites. Triabodies can be similarly constructed with three antigen-binding sites. An Fv fragment contains a complete antigen-binding site which includes a V\textsubscript{L} domain and a V\textsubscript{H} domain held together by non-covalent interactions. Fv fragments embraced by the present invention also include constructs in which the V\textsubscript{H} and V\textsubscript{L} domains are crosslinked through glutaraldehyde, intermolecular disulfides, or other linkers. The variable domains of the heavy and light chains can be fused together to form a single chain variable fragment (scFv), which retains the original specificity of the parent immunoglobulin. Single chain Fv (scFv) dimers, first described by Gruber et al, J. Immunol. 152(12):5368-74 (1994), may be constructed using heavy and light chains disclosed herein, as well as by using individual CDR regions disclosed herein. Many techniques known in the art can be used to prepare the specific binding constructs of the present invention (see, U.S. Patent Application Publication No. 20070196274 and U.S. Patent Application Publication No. 20050163782, which are each herein incorporated by reference in their entireties for all purposes, particularly with respect to minibody and diabody design).

Bispecific antibodies can be generated by chemical cross-linking or by the hybrid hybridoma technology. Alternatively, bispecific antibody molecules can be produced by recombinant techniques (see: bispecific antibodies). Dimersation can be promoted by
reducing the length of the linker joining the VH and the VL domain from about 15 amino acids, routinely used to produce scFv fragments, to about 5 amino acids. These linkers favor intrachain assembly of the VH and VL domains. A suitable short linker is SGGGS but other linkers can be used. Thus, two fragments assemble into a dimeric molecule. Further reduction of the linker length to 0-2 amino acids can generate trimeric (triabodies) or tetrameric (tetrabodies) molecules.

[0122] For preparation of suitable antibodies of the invention and for use according to the invention, e.g., recombinant, monoclonal, or polyclonal antibodies, many techniques known in the art can be used (see, e.g., Kohler & Milstein, *Nature* 256:495-497 (1975); Kozbor et al, *Immunology Today* 4: 72 (1983); Cole et al., pp. 77-96 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985); Coligan, *Current Protocols in Immunology* (1991); Harlow & Lane, *Antibodies, A Laboratory Manual* (1988); and Goding, *Monoclonal Antibodies: Principles and Practice* (2d ed. 1986)). The genes encoding the heavy and light chains of an antibody of interest can be cloned from a cell, e.g., the genes encoding a monoclonal antibody can be cloned from a hybridoma and used to produce a recombinant monoclonal antibody. Gene libraries encoding heavy and light chains of monoclonal antibodies can also be made from hybridoma or plasma cells. Random combinations of the heavy and light chain gene products generate a large pool of antibodies with different antigenic specificity (see, e.g., Kuby, *Immunology* (3rd ed. 1997)). Techniques for the production of single chain antibodies or recombinant antibodies (U.S. Patent 4,946,778, U.S. Patent No. 4,816,567) can be adapted to produce antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized or human antibodies (see, e.g., U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, Marks et al, *Bio/Technology* 10:779-783 (1992); Lonberg et al, *Nature* 368:856-859 (1994); Morrison, *Nature* 368:812-13 (1994); Fishwild et al, *Nature Biotechnology* 14:845-51 (1996); Neuberger, *Nature Biotechnology* 14:826 (1996); and Lonberg & Huszar, *Intern. Rev. Immunol.* 13:65-93 (1995)). Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens (see, e.g., McCafferty et al, *Nature* 348:552-554 (1990); Marks et al, *Biotechnology* 10:779-783 (1992)). Antibodies can also be made bispecific, i.e., able to recognize two different antigens (see, e.g., WO 93/08829, Traunecker et al, *EMBOJ.* 10:3655-3659 (1991); and Suresh et al, *Methods in Enzymology* 121:210 (1986)). Antibodies can also be heteroconjugates, e.g., two covalently joined antibodies, or
immunotoxins (see, e.g., U.S. Patent No. 4,676,980, WO 91/00360; WO 92/200373; and EP 03089).

[0123] Methods for humanizing or primatizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable domain. Humanization can be essentially performed following the method of Winter and co-workers (see, e.g., Jones et al, Nature 321:522-525 (1986); Riechmann et al, Nature 332:323-327 (1988); Verhoeyen et al, Science 239:1534-1536 (1988) and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0124] Suitable antibodies for use according to the invention are also disclosed in U.S. Patent Application Serial No. 12/590601, assigned to the same assignee as the present invention, and which is incorporated herein in its entirety and particularity further with respect to the humanized and other antibodies disclosed therein as well as the methods used in making them.

[0125] The ability of a particular antibody to recognize the same epitope as another antibody is typically determined by the ability of one antibody to competitively inhibit binding of the second antibody to the antigen, or a fragment or fusion thereof. Any of a number of competitive binding assays can be used to measure competition between two antibodies to the same antigen. An exemplary assay is a Biacore assay. Briefly in these assays, binding sites can be mapped in structural terms by testing the ability of interactants, e.g. different antibodies, to inhibit the binding of another. Injecting two consecutive antibody samples in sufficient concentration can identify pairs of competing antibodies for the same binding epitope. The antibody samples should have the potential to reach a significant saturation with each injection. The net binding of the second antibody injection is indicative for binding epitope analysis. Two response levels can be used to describe the boundaries of perfect competition versus non-competing binding due to distinct epitopes. The relative amount of binding response of the second antibody injection relative to the binding of
identical and distinct binding epitopes determines the degree of epitope overlap. Antibodies
may recognize linear or conformational epitopes, hence antibodies may be competitive while
recognizing dissimilar and distal epitopes.

[0126] Other conventional immunoassays known in the art can be used in the present
invention. For example, antibodies can be differentiated by the epitope to which they bind
using a sandwich ELISA assay. This is carried out by using a capture antibody to coat the
surface of a well. A subsaturating concentration of tagged-antigen is then added to the
capture surface. This protein will be bound to the antibody through a specific
antibody:epitope interaction. After washing a second antibody, which has been covalently
linked to a detectable moiety (e.g., HRP, with the labeled antibody being defined as the
detection antibody) is added to the ELISA. If this antibody recognizes the same epitope as the
capture antibody it will be unable to bind to the target protein as that particular epitope will
no longer be available for binding. If however this second antibody recognizes a different
epitope on the target protein it will be able to bind and this binding can be detected by
quantifying the level of activity (and hence antibody bound) using a relevant substrate. The
background is defined by using a single antibody as both capture and detection antibody,
whereas the maximal signal can be established by capturing with an antigen specific antibody
and detecting with an antibody to the tag on the antigen. By using the background and
maximal signals as references, antibodies can be assessed in a pair-wise manner to determine
epitope specificity.

[0127] A first antibody is considered to competitively inhibit binding of a second antibody,
if binding of the second antibody to the antigen is reduced by at least 30%, usually at least
about 40%, 50%, 60%> or 75%, and often by at least about 90%>, in the presence of the first
antibody using any of the assays described above.

[0128] A "chimeric antibody" is an antibody molecule in which (a) the constant region, or a
portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable
region) is linked to a constant region of a different or altered class, effector function and/or
species, or an entirely different molecule which confers new properties to the chimeric
antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable
region, or a portion thereof, is altered, replaced or exchanged with a variable region having a
different or altered antigen specificity. The preferred antibodies of, and for use according to
the invention include humanized and/or chimeric monoclonal antibodies.
In one embodiment, the antibody is conjugated to an "effector" moiety. The effector moiety can be any number of molecules, including labeling moieties such as radioactive labels or fluorescent labels, or can be a therapeutic moiety. In one aspect the antibody modulates the activity of the protein. Such effector moieties include, but are not limited to, an anti-tumor drug, a toxin, a radioactive agent, a cytokine, a second antibody or an enzyme. Further, the invention provides an embodiment wherein the antibody of the invention is linked to an enzyme that converts a prodrug into a cytotoxic agent.

The immunoconjugate can be used for targeting the effector moiety to a N-cadherin positive cell, particularly cells, which express the N-cadherin protein. Such differences can be readily apparent when viewing the bands of gels with approximately similarly loaded with test and controls samples. Examples of cytotoxic agents include, but are not limited to ricin, doxorubicin, daunorubicin, taxol, ethiduim bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, dihydroxy anthracin dione, actinomycin D, diptheria toxin, Pseudomonas exotoxin (PE) A, PE40, abrin, and glucocorticoid and other chemotherapeutic agents, as well as radioisotopes. Suitable detectable markers include, but are not limited to, a radioisotope, a fluorescent compound, a bioluminescent compound, chemiluminescent compound, a metal chelator or an enzyme.

In some embodiments, the invention provides antibodies to N-cadherin. N-cadherin antibodies may be used systemically to treat cancer (e.g., prostate or bladder cancer) alone or when conjugated with an effector moiety. N-cadherin antibodies conjugated with toxic agents, such as ricin, as well as unconjugated antibodies may be useful therapeutic agents naturally targeted to N-cadherin-bearing prostate cancer cells. Such antibodies can be useful in blocking invasiveness. Suitable N-cadherin antibodies for use according to the invention include, but are not limited to, EC4 1H7, 1F12, 2B3, as well as the antibodies disclosed in USSR 61/1 13,042 and 61/1 13,054, herein incorporated by reference in their entirety.

Additionally, the recombinant protein of the invention comprising the antigen-binding region of any of the monoclonal antibodies of the invention can be used to treat cancer. In such a situation, the antigen-binding region of the recombinant protein is joined to at least a functionally active portion of a second protein having therapeutic activity. The second protein can include, but is not limited to, an enzyme, lymphokine, oncostatin or toxin. Suitable toxins include doxorubicin, daunorubicin, taxol, ethiduim bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, dihydroxy anthracin dione,
actinomycin D, diptheria toxin, Pseudomonas exotoxin (PE) A, PE40, ricin, abrin, glucocorticoid and radioisotopes.


[0134] The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein, often in a heterogeneous population of proteins and other biologies. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and more typically more than 10 to 100 times background. Specific binding to an antibody under such conditions requires an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies can be selected to obtain only those polyclonal antibodies that are specifically immunoreactive with the selected antigen and not with other proteins. This selection may be achieved by subtracting out antibodies that cross-react with other molecules. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (see, e.g., Harlow & Lane, Using Antibodies, A Laboratory Manual (1998) for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity).

[0135] By "therapeutically effective dose or amount" herein is meant a dose that produces effects for which it is administered. The exact dose and formulation will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, e.g., Lieberman, Pharmaceutical Dosage Forms (vols. 1-3, 1992); Lloyd, The Art, Science and Technology of Pharmaceutical Compounding (1999); Remington: The Science and Practice of Pharmacy, 20th Edition, Gennaro, Editor (2003), and Pickar, Dosage Calculations (1999)).
The term "pharmaceutically acceptable salts" or "pharmaceutically acceptable carrier" is meant to include salts of the active compounds which are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds of the present invention contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present invention contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (see, e.g., Berge et al., Journal of Pharmaceutical Science 66:1-19 (1977)). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts. Other pharmaceutically acceptable carriers known to those of skill in the art are suitable for the present invention.

The neutral forms of the compounds may be regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present invention.

In addition to salt forms, the present invention provides compounds which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the present invention. Additionally, prodrugs can be converted to the compounds of the present invention by chemical or biochemical methods in an ex vivo environment. For example,
prodrugs can be slowly converted to the compounds of the present invention when placed in a transdermal patch reservoir with a suitable enzyme or chemical reagent.

[0139] Certain compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are intended to be encompassed within the scope of the present invention. Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

[0140] Certain compounds of the present invention possess asymmetric carbon atoms (optical centers) or double bonds; the racemates, diastereomers, geometric isomers and individual isomers are all intended to be encompassed within the scope of the present invention.

[0141] Epithelial to Mesenchymal Transition (EMT) refers to the acquisition of stromal features by epithelial tumor cells. In cancer, EMT is associated with invasive and motile behavior and may be central process underlying metastasis. EMT is associated with poor prognosis and is mediated by multiple transcription factors, such as, SNAIL, SLUG and TWIST.

III. Compositions and Methods

[0142] In one aspect, the present invention provides an N-cadherin antibody, or a pharmaceutical composition comprising a pharmaceutically acceptable excipient and an N-cadherin antibody, wherein the N-cadherin antibody binds to the N-cadherin epitope SDPANWLKIDPVNG (SEQ ID NO: 10) or any portion thereof. In some embodiments, the antibody competes with EC4 for binding to N-cadherin or binds N-cadherin within the sequence SDPANWLKIDPVNGQITTTIAVL (SEQ ID NO: 17) or QQNIRYTKLSDPANWLKIDPVNGQITTTIAVL (SEQ ID NO: 18). In some embodiments, the antibody is a monoclonal antibody, an scFv fragment, a diabody, a minibody, or a triabody. In some embodiments, the antibody is a humanized antibody. In some embodiments, the antibody is linked to a therapeutic agent, a diagnostic agent, or a detectable label.

[0143] In another aspect, the present invention provides methods of diagnosis and providing a prognosis for individuals at risk for a cancer that expresses a N-cadherin protein or mPvNA transcript, particularly urogenital cancers including prostate and/or bladder cancer. The methods generally comprise contacting a test tissue sample from an individual at risk of
having a cancer that expresses a N-cadherin protein or mRNA transcript with an antibody that specifically binds to a N-cadherin protein; and determining the presence or absence of a N-cadherin protein in the test tissue sample in comparison to a control tissue sample from an individual known to be negative for a cancer that expresses a N-cadherin protein or mRNA transcript. In some embodiments, the methods comprise contacting the test tissue sample with an antibody (e.g., a monoclonal antibody, an scFv fragment, a diabody, a minibody, or a triabody) that binds to the N-cadherin epitope SDPANWLKIDPVNG (SEQ ID NO: 10) or any portion thereof. In some embodiments, the methods comprise contacting the test tissue sample with an antibody (e.g., a monoclonal antibody, a diabody, a minibody, or a triabody) that competes with EC4 for binding to N-cadherin or binds N-cadherin within the sequence SDPANWLKIDPVNGQITTIAVLD (SEQ ID NO: 17) or QQNIRYTKLSDPANWLKIDPVNGQITTIAVLD (SEQ ID NO: 18). Typically, the tissue sample is serum, but can also be a tissue from a biopsy, particularly from a urogenital tissue including prostate tissue or bladder tissue. A positive diagnosis for a cancer that expresses a N-cadherin protein or mRNA transcript is indicated when a higher level of N-cadherin protein is detected in a test tissue sample in comparison to a control tissue sample from an individual known not to have cancer, for example, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 2-fold, 3-fold, 4-fold higher or more. The detection methods can be carried out, for example, using standard ELISA techniques known in the art (reviewed in Gosling, Immunoassays: A Practical Approach, 2000, Oxford University Press). Detection is accomplished by labeling a primary antibody or a secondary antibody with, for example, a radioactive isotope, a fluorescent label, an enzyme or any other detectable label known in the art.

[0144] In another embodiment, invention provides methods of diagnosis and providing a prognosis for individuals at risk for a cancer that expresses a N-cadherin protein or mRNA transcript, particularly a prostate or bladder cancer, by contacting a test tissue sample from an individual at risk of having a cancer that expresses a N-cadherin protein or mRNA transcript with a primer set of a first oligonucleotide and a second oligonucleotide that each specifically hybridize to a N-cadherin nucleic acid; amplifying the N-cadherin nucleic acid in the sample; and determining the presence or absence of the N-cadherin nucleic acid in the test tissue sample in comparison to a control tissue sample from an individual known to be negative for a cancer that expresses a N-cadherin protein or mRNA transcript. Again, usually the tissue sample is serum, but can also be a tissue from a biopsy, particularly a urogenital tissue including a prostate or bladder tissue. A positive diagnosis for a cancer that expresses a N-
cadherin protein or mRNA transcript is indicated when a higher level of N-cadherin transcribed RNA is detected in a test tissue sample in comparison to a control tissue sample from an individual known not to have cancer.

[0145] The invention also provides methods for improving the response to cancer therapy in a cancer that expresses a N-cadherin protein or mRNA transcript by administering a therapeutically effective amount of a compound that inhibits the binding of N-cadherin protein to a N-cadherin receptor on a cell of the cancer tumor tissue. In some embodiments, the compound is an antibody (e.g., a monoclonal antibody, an scFv fragment, a diabody, a minibody, or a triabody) that binds to the N-cadherin epitope SDPANWLKIDPVNG (SEQ ID NO: 10) or any portion thereof. In some embodiments, the compound is an antibody that competes with EC4 for binding to N-cadherin or binds N-cadherin within the sequence SDPANWLKIDPVNGQITTIAVLD (SEQ ID NO: 17) or QQNIRYTKLSDPANWLKIDPVNGQITTIAVLD (SEQ ID NO: 18). In some embodiments the methods of inhibiting N-cadherin binding to its receptor are carried out concurrently with another anticancer therapy, including, for example, known chemotherapeutics, immunotherapeutics, and radiotherapy for the reversal of resistance, tumor progression, and metastasis.

[0146] The present invention further provides methods of inhibiting the growth of and promoting the regression of a tumor that expresses N-cadherin protein, the methods comprising inhibiting the binding of N-cadherin protein to a N-cadherin receptor on a cell of the tumor tissue. The methods find particular use in treating prostate and bladder cancer. The methods can be carried out by administering to an individual in need thereof a sufficient amount of a compound that inhibits the binding of a N-cadherin protein to a N-cadherin receptor. In some embodiments, the compound specifically binds to a N-cadherin protein. In some embodiments, the compound prevents the transcription or the translation of a N-cadherin protein. In some embodiments, the compound comprises a polypeptide, including an antibody or an analog or fragment of a N-cadherin polypeptide. In some embodiments, the compound is an antibody (e.g., a monoclonal antibody, an scFv fragment, a diabody, a minibody, or a triabody) that binds to the N-cadherin epitope SDPANWLKIDPVNG (SEQ ID NO: 10) or any portion thereof. In some embodiments, the compound is an antibody that competes with EC4 for binding to N-cadherin or binds N-cadherin within the sequence SDPANWLKIDPVNGQITTIAVLD (SEQ ID NO: 17) or QQNIRYTKLSDPANWLKIDPVNGQITTIAVLD (SEQ ID NO: 18).
The methods described herein find particular application in the diagnosis, prognosis and treatment of prostate and bladder cancers. In certain embodiments the methods are applied to hormone refractory or therapy resistant cancers. In certain embodiments the methods are applied to metastatic cancers. For example comparisons of differential expression of a N-cadherin protein and/or mRNA can be used to determine the stage of cancer of an individual having a cancer that expresses a N-cadherin protein or mRNA transcript.

Treatment will generally involve the repeated administration of the anti-N-cadherin antibodies, immunoconjugates, inhibitors, and siRNA preparations via an acceptable route of administration such as intravenous injection (IV), at an effective dose. Dosages will depend upon various factors generally appreciated by those of skill in the art, including without limitation the type of cancer and the severity, grade, or stage of the cancer, the binding affinity and half life of the agents used, the degree of N-cadherin expression in the patient, the extent of circulating shed N-cadherin antigen, the desired steady-state antibody concentration level, frequency of treatment, and the influence of chemotherapeutic agents used in combination with the treatment method of the invention. Typical daily doses may range from about 0.1 to 100 mg/kg. Doses in the range of 10-500 mg of the mAb or immunoconjugates per week may be effective and well tolerated, although even higher weekly doses may be appropriate and/or well tolerated. The principal determining factor in defining the appropriate dose is the amount of a particular agent necessary to be therapeutically effective in a particular context. Repeated administrations may be required in order to achieve tumor inhibition or regression. Initial loading doses may be higher. The initial loading dose may be administered as an infusion. Periodic maintenance doses may be administered similarly, provided the initial dose is well tolerated.

Direct administration of the agents is also possible and may have advantages in certain contexts. For example, for the treatment of bladder carcinoma, the agents may be injected directly into the bladder. Because agents administered directly to bladder will be cleared from the patient rapidly, it may be possible to use non-human or chimeric antibodies effectively without significant complications of antigenicity.

The invention further provides vaccines formulated to contain a N-cadherin protein or fragment thereof and N-cadherin polypeptides disclosed herein. In some embodiments, the vaccine comprises a polypeptide consisting of at least 24 contiguous amino acids of the sequence QQNIRYTKLSDPANWLKIDPVNGQITTIAVLD (SEQ ID NO: 18). In some embodiments, the vaccine comprises a polypeptide consisting at least 14, 15, 16, 17, 20, 25 or
30 contiguous amino acids of N-cadherin and including the sequence SDPANWLKIDPVNG (SEQ ID NO: 10). In some embodiments, the vaccine comprises a polypeptide having the sequence SDPANWLKIDPVNG (SEQ ID NO: 10). The use of a tumor antigen in a vaccine for generating humoral and cell-mediated immunity for use in anti-cancer therapy is well known in the art and, for example, has been employed in prostate cancer using human PSMA and rodent PAP immunogens (Hodge et al, 1995, Int. J. Cancer 63: 231-237; Fong et al, 1997, J. Immunol. 159: 3113-3117). Such methods can be readily practiced by employing a N-cadherin protein, or fragment thereof, or a N-cadherin -encoding nucleic acid molecule and recombinant vectors capable of expressing and appropriately presenting the N-cadherin immunogen.

[0151] For example, viral gene delivery systems may be used to deliver a N-cadherin encoding nucleic acid molecule. Various viral gene delivery systems which can be used in the practice of this aspect of the invention include, but are not limited to, vaccinia, fowlpox, canarypox, adenovirus, influenza, poliovirus, adeno-associated virus, lentivirus, and sindbis virus (Restifo, 1996, Curr. Opin. Immunol. 8: 658-663). Non-viral delivery systems may also be employed by using naked DNA encoding a N-cadherin protein or fragment thereof introduced into the patient (e.g., intramuscularly) to induce an anti-tumor response. In one embodiment, the full-length human N-cadherin cDNA may be employed. In another embodiment, N-cadherin nucleic acid molecules encoding specific cytotoxic T lymphocyte (CTL) epitopes may be employed. CTL epitopes can be determined using specific algorithms (e.g., Epimer, Brown University) to identify peptides within a N-cadherin protein which are capable of optimally binding to specified HLA alleles.

[0152] Various ex vivo strategies may also be employed. One approach involves the use of dendritic cells to present N-cadherin antigen to a patient's immune system. Dendritic cells express MHC class I and II, B7 costimulator, and IL-12, and are thus highly specialized antigen presenting cells. In prostate cancer, autologous dendritic cells pulsed with peptides of the N-cadherin can be used to stimulate prostate cancer patients' immune systems (Tjoa et al., 1996, Prostate 28: 65-69; Murphy et al., 1996, Prostate 29: 371-380). Dendritic cells can be used to present N-cadherin peptides to T cells in the context of MHC class I and II molecules. In one embodiment, autologous dendritic cells are pulsed with N-cadherin peptides capable of binding to MHC molecules. In another embodiment, dendritic cells are pulsed with the complete N-cadherin protein. Yet another embodiment involves engineering the expression of the N-cadherin gene in dendritic cells using various implementing vectors known in the art, such as adenovirus (Arthur et al, 1997, Cancer Gene Ther. 4: 17-25), retrovirus

[0153] Anti-idiotypic anti-N-cadherin antibodies can also be used in anti-cancer therapy as a vaccine for inducing an immune response to cells expressing a N-cadherin protein, respectively. Specifically, the generation of anti-idiotypic antibodies is well known in the art and can readily be adapted to generate anti-idiotypic anti-N-cadherin antibodies that respectively mimic an epitope on a N-cadherin protein (see, for example, Wagner et al., 1997, Hybridoma 16: 33-40; Foon et al., 1995, J Clin Invest 96: 334-342; Herlyn et al., 1996, Cancer Immunol Immunother 43: 65-76). Such an anti-idiotypic antibody can be used in anti-idiotypic therapy as presently practiced with other anti-idiotypic antibodies directed against tumor antigens.

[0154] Genetic immunization methods may be employed to generate prophylactic or therapeutic humoral and cellular immune responses directed against cancer cells expressing N-cadherin. Using the N-cadherin -encoding DNA molecules described herein, constructs comprising DNA encoding a N-cadherin protein/immunogen and appropriate regulatory sequences may be injected directly into muscle or skin of an individual, such that the cells of the muscle or skin take-up the construct and express the encoded N-cadherin protein/immunogen. The N-cadherin protein/immunogen may be expressed as a cell surface protein or be secreted. Expression of the N-cadherin protein/immunogen results in the generation of prophylactic or therapeutic humoral and cellular immunity against prostate cancer. Various prophylactic and therapeutic genetic immunization techniques known in the art may be used (for review, see information and references published at internet address www.genweb.com).

[0155] The invention further provides methods for inhibiting cellular activity (e.g., cell proliferation, activation, or propagation) of a cell expressing multiple N-cadherin antigens on its cell surface. This method comprises reacting the immunoconjugates of the invention (e.g., a heterogeneous or homogenous mixture) with the cell so that the N-cadherin antigens on the cell surface forms a complex with the immunoconjugates. The greater the number of N-cadherin antigens on the cell surface, the greater the number of N-cadherin -antibody complexes that can, respectively, be used. The greater the number of N-cadherin -antibody complexes the greater the cellular activity that is inhibited.
[0156] A heterogeneous mixture includes N-cadherin antibodies that recognize different or the same epitope, each antibody being conjugated to the same or different therapeutic agent. A homogenous mixture includes antibodies that recognize the same epitope, each antibody being conjugated to the same therapeutic agent. In some embodiments, the epitope is SDPANWLKIDPVNG (SEQ ID NO: 10) or any portion thereof.

[0157] The invention further provides methods for inhibiting the biological activity of N-cadherin by respectively blocking N-cadherin from binding its receptor. The methods comprises contacting an amount of N-cadherin with an antibody or immunoconjugate of the invention under conditions that permit a N-cadherin-immunoconjugate or N-cadherin-antibody complex thereby, respectively, blocking N-cadherin from binding its ligand and inhibiting the activity of N-cadherin. In some embodiments, the method comprises contacting an amount of N-cadherin with an antibody (e.g., a monoclonal antibody, an scFv fragment, a diabody, a minibody, or a triabody) that binds to the N-cadherin epitope SDPANWLKIDPVNG (SEQ ID NO: 10) or any portion thereof. In some embodiments, the method comprises contacting an amount of N-cadherin with an antibody (e.g., a monoclonal antibody, a diabody, a minibody, or a triabody) that competes with EC4 for binding to N-cadherin or binds N-cadherin within the sequence SDPANWLKIDPVNGQITTIAVLD (SEQ ID NO:17) or QQNIRYTKLSDPANWLKIDPVNGQITTIAVLD (SEQ ID NO:18).

[0158] In some embodiments, the invention provides a method of treating cancer, particularly a cancer which expresses N-cadherin, or of inhibiting the growth of a cancer cell expressing a N-cadherin protein by treating a subject or contacting the cancer cell with an antibody or fragment thereof that recognizes and binds the N-cadherin protein in an amount effective to inhibit the growth of the cancer cell. In some embodiments, the method comprises treating the subject or contacting the cancer cell with an antibody (e.g., a monoclonal antibody, an scFv fragment, a diabody, a minibody, or a triabody) that binds to the N-cadherin epitope SDPANWLKIDPVNG (SEQ ID NO: 10) or any portion thereof. In some embodiments, the method comprises contacting the subject or contacting the cancer cell with an antibody (e.g., a monoclonal antibody, a diabody, a minibody, or a triabody) that competes with EC4 for binding to N-cadherin or binds N-cadherin within the sequence SDPANWLKIDPVNGQITTIAVLD (SEQ ID NO: 17) or QQNIRYTKLSDPANWLKIDPVNGQITTIAVLD (SEQ ID NO: 18). In some embodiments, the cancer cell is a prostate cancer cell or a bladder cancer cell. The contacting antibody can be a monoclonal antibody and/or a chimeric antibody. In some embodiments, the chimeric antibody comprises a human immunoglobulin constant region. In some embodiments, the
antibody is a human antibody or comprises a human immunoglobulin constant region. In further embodiments, the antibody fragment comprises an Fab, F(ab)_2, or Fv. In other embodiments, the fragment comprises a recombinant protein having an antigen-binding region.

[0159] In another embodiment, the invention provides methods for treating cancer, particularly, a cancer expressing N-cadherin, or inhibiting the growth of a cancer cell expressing an N-cadherin protein by treating the subject or contacting the cancer cell with an immunoconjugate comprising an antibody that binds to the N-cadherin epitope SDPANWLKIDPVNG (SEQ ID NO: 10) or any portion thereof in an amount sufficient to treat the cancer or inhibit the growth of the cell. Such amounts include an amount to kill the cell or an amount sufficient to inhibit cell growth or proliferation. As discussed infra, the dose and dosage regimen will depend on the nature of the disease or disorder to be treated associated with N-cadherin, its population, the site to which the antibodies are to be directed, the characteristics of the particular immunotoxin, and the patient. For example, the amount of immunoconjugate can be in the range of 0.1 to 200 mg/kg of patient weight. The immunoconjugate can comprise the anti-N-cadherin antibody or the fragment linked to a therapeutic agent. The therapeutic agent can be a cytotoxic agent. The cytotoxic agent can be selected from a group consisting of ricin, ricin A-chain, doxorubicin, daunorubicin, taxol, ethidium bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, dihydroxy anthracin dione, actinomycin D, diptheria toxin, Pseudomonas exotoxin (PE) A, PE40, abrin, abrin A chain, modeccin A chain, alpha-sarcin, gelonin mitogellin, retstrictocin, phenomycin, enomycin, curcin, crotin, calicheamicin, sapaonaria officinalis inhibitor, maytansinoids, and glucocorticoid. The therapeutic agent can be a radioactive isotope. The therapeutic isotope can be selected from the group consisting of ^{212}Bi, ^{131}I, ^{111}In, ^{90}Y and ^{186}Re.

[0160] In any of the embodiments above, a chemotherapeutic drug and/or radiation therapy can be administered further. In some embodiments, the patient also receives hormone antagonist therapy. The contacting of the patient with the antibody or antibody fragment, can be by administering the antibody to the patient intravenously, intraperitoneally, intramuscularly, intratumorally, or intradermally. In some embodiments, the patient has a urogenital cancer (e.g., bladder cancer, prostate cancer). In some embodiments of the above, the patient suffers from prostate cancer and optionally further receives patient hormone ablation therapy. In some embodiments, the contacting comprises administering the antibody directly into the cancer or a metastasis of the cancer.
In some embodiments, the immunoconjugate has a cytotoxic agent which is a small molecule. Toxins such as maytansin, maytansinoids, saporin, gelonin, ricin or calicheamicin and analogs or derivatives thereof are also suitable. Other cytotoxic agents that can be conjugated to the anti-N-cadherin antibodies include BCNU, streptozocin, vincristine and 5-fluorouracil. Enzymatically active toxins and fragments thereof can also be used. The radio-effector moieties may be incorporated in the conjugate in known ways (e.g., bifunctional linkers, fusion proteins). The antibodies of the present invention may also be conjugated to an effector moiety which is an enzyme which converts a prodrug to an active chemotherapeutic agent. See, WO 88/07378; U. S. Patent No. 4,975,278; and U.S. Patent No. 6,949,245. The antibody or immunoconjugate may optionally be linked to nonprotein polymers (e.g., polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol).

Conjugates of the antibody and cytotoxic agent may be made using methods well known in the art (see, U.S. Patent No. 6,949,245). For instance, the conjugates may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azido benzoyl) hexanediame), bis-diazenium derivatives (such as bis-(p-diazenium benzoyl)-ethylendiamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al. Science 238: 1098 (1987). Carbon-14-labeled l-isothiocyanatobenzyl-3-methyl diethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See W094/1 1026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, dimethyl linker or disulfide-containing linker (Chari et al. Cancer Research 52: 127-131 (1992)) may be used.

IV. Methods of Administration and Formulation

The anti-N-cadherin antibodies or immunoconjugates are administered to a human patient in accord with known methods, such as intravenous administration, e.g., as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or
inhalation routes. Intravenous or subcutaneous administration of the antibody is preferred. The administration may be local or systemic.

[0164] The compositions for administration will commonly comprise an agent as described herein (e.g., N-cadherin inhibitors, N-cadherin antibodies and immunoconjugates, N-cadherin siRNA and vectors thereof) dissolved in a pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., buffered saline and the like. These solutions are sterile and generally free of undesirable matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of active agent in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient's needs.

[0165] Thus, a typical pharmaceutical composition for intravenous administration will vary according to the agent. Actual methods for preparing parenterally administerable compositions will be known or apparent to those skilled in the art and are described in more detail in such publications as Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pa. (1980).

[0189] The pharmaceutical compositions can be administered in a variety of unit dosage forms depending upon the method of administration. For example, unit dosage forms suitable for oral administration include, but are not limited to, powder, tablets, pills, capsules and lozenges. It is recognized that antibodies when administered orally, should be protected from digestion. This is typically accomplished either by complexing the molecules with a composition to render them resistant to acidic and enzymatic hydrolysis, or by packaging the molecules in an appropriately resistant carrier, such as a liposome or a protection barrier. Means of protecting agents from digestion are well known in the art.

[0166] Pharmaceutical formulations, particularly, of the antibodies and immunoconjugates and inhibitors for use with the present invention can be prepared by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers. Such formulations can be lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the
dosages and concentrations used. Acceptable carriers, excipients or stabilizers can be acetate, phosphate, citrate, and other organic acids; antioxidants (e.g., ascorbic acid) preservatives low molecular weight polypeptides; proteins, such as serum albumin or gelatin, or hydrophilic polymers such as polyvinylpyrrolidone; and amino acids, monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents; and ionic and non-ionic surfactants (e.g., polysorbate); salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants. The antibody can be formulated at a concentration of between 0.5 - 200 mg/ml, or between 10-50 mg/ml.

[0167] The formulation may also provide additional active compounds, including, chemotherapeutic agents, cytotoxic agents, cytokines, growth inhibitory agent, and anti-hormonal agent. The active ingredients may also prepared as sustained-release preparations (e.g., semi-permeable matrices of solid hydrophobic polymers (e.g., polyesters, hydrogels (for example, poly (2-hydroxyethyl-methacrylate), or poly (vinylalcohol)) polylactides. The antibodies and immunoconjugates may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin microcapsules and poly- (methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions.

[0168] The compositions can be administered for therapeutic or prophylactic treatments. In therapeutic applications, compositions are administered to a patient suffering from a disease (e.g., cancer) in a "therapeutically effective dose." Amounts effective for this use will depend upon the severity of the disease and the general state of the patient's health. Single or multiple administrations of the compositions may be administered depending on the dosage and frequency as required and tolerated by the patient. A "patient" or "subject" for the purposes of the present invention includes both humans and other animals, particularly mammals. Thus the methods are applicable to both human therapy and veterinary applications. In the preferred embodiment the patient is a mammal, preferably a primate, and in the most preferred embodiment the patient is human. Other known cancer therapies can be used in combination with the methods of the invention. For example, the compositions for use according to the invention may also be used to target or sensitize a cell to other cancer therapeutic agents such as 5FU, vinblastine, actinomycin D, cisplatin, methotrexate, and the like.
In other embodiments, the methods of the invention with other cancer therapies (e.g., radical prostatectomy), radiation therapy (external beam or brachytherapy), hormone therapy (e.g., orchietomy, LHRH-analog therapy to suppress testosterone production, anti-androgen therapy), or chemotherapy. Radical prostatectomy involves removal of the entire prostate gland plus some surrounding tissue. This treatment is used commonly when the cancer is thought not to have spread beyond the tissue. Radiation therapy is commonly used to treat prostate cancer that is still confined to the prostate gland, or has spread to nearby tissue. If the disease is more advanced, radiation may be used to reduce the size of the tumor. Hormone therapy is often used for patients whose prostate cancer has spread beyond the prostate or has recurred. The objective of hormone therapy is to lower levels of the male hormones, androgens and thereby cause the prostate cancer to shrink or grow more slowly. Luteinizing hormone-releasing hormone (LHRH) agonists decrease the production of testosterone. These agents may be injected either monthly or longer. Two such analogs are leuprolide and goserelin. Anti-androgens (e.g., flutamide, bicalutamide, and nilutamide) may also be used. Total androgen blockade, which refers to the use of anti-androgens in combination with orchietomy or LHRH analogs, may also be used. Chemotherapy is an option for patients whose prostate cancer has spread outside of the prostate gland and for whom hormone therapy has failed. It is not expected to destroy all of the cancer cells, but it may slow tumor growth and reduce pain. Some of the chemotherapy drugs used in treating prostate cancer that has returned or continued to grow and spread after treatment with hormonal therapy include doxorubicin (Adriamycin), estramustine, etoposide, mitoxantrone, vinblastine, and paclitaxel. Two or more drugs are often given together to reduce the likelihood of the cancer cells becoming resistant to chemotherapy. Small cell carcinoma is a rare type of prostate cancer that is more likely to respond to chemotherapy than to hormonal therapy.

In some embodiments, a "cardioprotectant" is also administered with the N-cadherin antibody, N-cadherin binding inhibitor, or N-cadherin siRNA molecule for use to according to the invention (see, U.S. Patent No. 6,949,245). A cardioprotectant is a compound or composition which prevents or reduces myocardial dysfunction (i.e. cardiomyopathy and/or congestive heart failure) associated with administration of a drug, such as an anthracycline antibiotic to a patient. The cardioprotectant may, for example, block or reduce a free-radical-mediated cardiotoxic effect and/or prevent or reduce oxidative-stress injury. Examples of cardioprotectants encompassed by the present definition include the iron-chelating agent dexrazoxane (ICRF-187) (Seifert et al. The Annals of Pharmacotherapy 28:1063-1072 (1994)); a lipid-lowering agent and/or anti-oxidant such as probucol (Singal et al. J. Mol. Cell
Cardiol. 27:1055-1063 (1995); amifostine (aminothiol 2-[(3-
aminopropyl)amino]ethanethiol-dihydrogen phosphate ester, also called WR-2721, and the
dephosphorylated cellular uptake form thereof called WR-1065) and S-3-(3-
methylaminopropylamino)propylphosphorothioic acid (WR-151327), see Green et al.
Cancer Research 54:738-741 (1994); digoxin (Bristow, M. R. In: Bristow M R, ed. Drug-
Induced Heart Disease. New York: Elsevier 191-215 (1980)); beta-blockers such as
129:197-9 (1995)); vitamin E; ascorbic acid (vitamin C); free radical scavengers such as
oleanolic acid, ursolic acid and N-acetylcysteine (NAC); spin trapping compounds such as
alpha-phenyl-tert-butyl nitrone (PBN); (Paracchini et al., Anticancer Res. 13:1607-1612
(1993)); selenoorganic compounds such as P251 (Elbesen); and the like.

[0171] The combined administrations contemplates coadministration, using separate
formulations or a single pharmaceutical formulation, and consecutive administration in either
order, wherein preferably there is a time period while both (or all) active agents
simultaneously exert their biological activities.

[0172] Molecules and compounds identified that indirectly or directly modulate the
expression and/or function of a N-cadherin protein can be useful in treating cancers that
express N-cadherin. N-cadherin protein modulators can be administered alone or co-
administered in combination with conventional chemotherapy, radiotherapy or
immunotherapy as well as currently developed therapeutics.

[0173] Formulations suitable for oral administration can consist of (a) liquid solutions, such
as an effective amount of the packaged nucleic acid suspended in diluents, such as water,
saline or PEG 400; (b) capsules, sachets or tablets, each containing a predetermined amount
of the active ingredient, as liquids, solids, granules or gelatin; (c) suspensions in an
appropriate liquid; and (d) suitable emulsions. Tablet forms can include one or more of
lactose, sucrose, mannitol, sorbitol, calcium phosphates, corn starch, potato starch,
microcrystalline cellulose, gelatin, colloidal silicon dioxide, talc, magnesium stearate, stearic
acid, and other excipients, colorants, fillers, binders, diluents, buffering agents, moistening
agents, preservatives, flavoring agents, dyes, disintegrating agents, and pharmaceutically
compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, e.g.,
sucrose, as well as pastilles comprising the active ingredient in an inert base, such as gelatin
and glycerin or sucrose and acacia emulsions, gels, and the like containing, in addition to the
active ingredient, carriers known in the art.
The compound of choice, alone or in combination with other suitable components, can be made into aerosol formulations (i.e., they can be "nebulized") to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

Suitable formulations for rectal administration include, for example, suppositories, which consist of the packaged nucleic acid with a suppository base. Suitable suppository bases include natural or synthetic triglycerides or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules which consist of a combination of the compound of choice with a base, including, for example, liquid triglycerides, polyethylene glycols, and paraffin hydrocarbons.

Formulations suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous, intramuscular, intratumoral, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions can be administered, for example, by intravenous infusion, orally, topically, intraperitoneally, intravesically or intrathecally. Parenteral administration, oral administration, and intravenous administration are the preferred methods of administration. The formulations of compounds can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials.

Injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. Cells transduced by nucleic acids for ex vivo therapy can also be administered intravenously or parenterally as described above.

The pharmaceutical preparation is preferably in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form. The composition can, if desired, also contain other compatible therapeutic agents.

Preferred pharmaceutical preparations deliver one or more active N-cadherin protein modulators, optionally in combination with one or more chemotherapeutic agents or
immunotherapeutic agents, in a sustained release formulation. Typically, the N-cadherin modulator is administered therapeutically as a sensitizing agent that increases the susceptibility of tumor cells to other cytotoxic cancer therapies, including chemotherapy, radiation therapy, immunotherapy and hormonal therapy.

[0180] In therapeutic use for the treatment of cancer, the N-cadherin modulators or inhibitors utilized in the pharmaceutical method of the invention are administered at the initial dosage of about 0.001 mg/kg to about 1000 mg/kg daily. A daily dose range of about 0.01 mg/kg to about 500 mg/kg, or about 0.1 mg/kg to about 200 mg/kg, or about 1 mg/kg to about 100 mg/kg, or about 10 mg/kg to about 50 mg/kg, can be used. The dosages, however, may be varied depending upon the requirements of the patient, the severity of the condition being treated, and the compound being employed. For example, dosages can be empirically determined considering the type and stage of cancer diagnosed in a particular patient. The dose administered to a patient, in the context of the present invention should be sufficient to effect a beneficial therapeutic response in the patient over time. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular vector, or transduced cell type in a particular patient. Determination of the proper dosage for a particular situation is within the skill of the practitioner. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day, if desired.

[0181] The pharmaceutical preparations (e.g., N-cadherin siRNAs, N-cadherin antibodies, N-cadherin vaccines, N-cadherin inhibitors, and immunoconjugates) for use according to the invention are typically delivered to a mammal, including humans and non-human mammals. Non-human mammals treated using the present methods include domesticated animals (i.e., canine, feline, murine, rodentia, and lagomorpha) and agricultural animals (bovine, equine, ovine, porcine).

V. Assays for Modulators of N-cadherin protein

[0182] Modulation of a N-Cadherin protein, and corresponding modulation of cellular, e.g., tumor cell, proliferation, can be assessed using a variety of in vitro and in vivo assays, including cell-based models. Such assays can be used to test for inhibitors and activators of a N-Cadherin protein, and, consequently, inhibitors and activators of cellular proliferation, including modulators of chemotherapeutic sensitivity and toxicity. Such modulators of a N-
Cadherin protein are useful for treating disorders related to pathological cell proliferation, e.g., cancer. Modulators of N-Cadherin protein are tested using either recombinant or naturally occurring N-Cadherin, preferably human N-Cadherin.

[0183] Measurement of cellular proliferation modulation with a N-Cadherin protein or a cell expressing a N-Cadherin protein, either recombinant or naturally occurring, can be performed using a variety of assays, in vitro, in vivo, and ex vivo, as described herein. A suitable physical, chemical or phenotypic change that affects activity, e.g., enzymatic activity such as kinase activity, cell proliferation, or ligand binding (e.g., a N-Cadherin protein receptor) can be used to assess the influence of a test compound on the polypeptide of this invention. When the functional effects are determined using intact cells or animals, one can also measure a variety of effects, such as, ligand binding, kinase activity, transcriptional changes to both known and uncharacterized genetic markers (e.g., northern blots), changes in cell metabolism, changes related to cellular proliferation, cell surface marker expression, DNA synthesis, marker and dye dilution assays (e.g., GFP and cell tracker assays), contact inhibition, tumor growth in nude mice, etc.

A. In vitro assays

[0184] Assays to identify compounds with N-cadherin modulating activity can be performed in vitro. Such assays can use a full length N-cadherin protein or a variant thereof (see, e.g., Figures 33 and 34, respectively), or a mutant thereof, or a fragment of a N-cadherin protein. Purified recombinant or naturally occurring N-cadherin protein can be used in the in vitro methods of the invention. In addition to purified N-cadherin protein, the recombinant or naturally occurring N-cadherin protein can be part of a cellular lysate or a cell membrane. As described below, the binding assay can be either solid state or soluble. Preferably, the protein or membrane is bound to a solid support, either covalently or non-covalently. Often, the in vitro assays of the invention are substrate or ligand binding or affinity assays, either non-competitive or competitive. Other in vitro assays include measuring changes in spectroscopic (e.g., fluorescence, absorbance, refractive index), hydrodynamic (e.g., shape), chromatographic, or solubility properties for the protein. Other in vitro assays include enzymatic activity assays, such as phosphorylation or autophosphorylation assays).

[0185] In one embodiment, a high throughput binding assay is performed in which the N-cadherin protein or a fragment thereof is contacted with a potential modulator and incubated for a suitable amount of time. In one embodiment, the potential modulator is bound to a solid support, and the N-cadherin protein is added. In another embodiment, the N-cadherin protein
is bound to a solid support. A wide variety of modulators can be used, as described below, including small organic molecules, peptides, antibodies, and N-cadherin ligand analogs. A wide variety of assays can be used to identify N-cadherin-modulator binding, including labeled protein-protein binding assays, electrophoretic mobility shifts, immunoassays, enzymatic assays such as kinase assays, and the like. In some cases, the binding of the candidate modulator is determined through the use of competitive binding assays, where interference with binding of a known ligand or substrate is measured in the presence of a potential modulator.

[0186] In one embodiment, microtiter plates are first coated with either a N-cadherin protein or a N-cadherin protein receptor, and then exposed to one or more test compounds potentially capable of inhibiting the binding of a N-cadherin protein to a N-cadherin protein receptor. A labeled (i.e., fluorescent, enzymatic, radioactive isotope) binding partner of the coated protein, either a N-cadherin protein receptor or a N-cadherin protein, is then exposed to the coated protein and test compounds. Unbound protein is washed away as necessary in between exposures to a N-cadherin protein, a N-cadherin protein receptor, or a test compound. An absence of detectable signal indicates that the test compound inhibited the binding interaction between a N-cadherin protein and, respectively, a N-cadherin protein receptor. The presence of detectable signal (i.e., fluorescence, colorimetric, radioactivity) indicates that the test compound did not inhibit the binding interaction between a N-cadherin protein and, respectively, a N-cadherin protein receptor. The presence or absence of detectable signal is compared to a control sample that was not exposed to a test compound, which exhibits uninhibited signal. In some embodiments the binding partner is unlabeled, but exposed to a labeled antibody that specifically binds the binding partner.

B. Cell-based in vivo assays

[0187] In another embodiment, N-cadherin protein is expressed in a cell, and functional, e.g., physical and chemical or phenotypic, changes are assayed to identify N-cadherin and modulators of cellular proliferation, e.g., tumor cell proliferation. Cells expressing N-cadherin proteins can also be used in binding assays and enzymatic assays. Any suitable functional effect can be measured, as described herein. For example, cellular morphology (e.g., cell volume, nuclear volume, cell perimeter, and nuclear perimeter), ligand binding, kinase activity, apoptosis, cell surface marker expression, cellular proliferation, GFP positivity and dye dilution assays (e.g., cell tracker assays with dyes that bind to cell membranes), DNA synthesis assays (e.g., ³H-thymidine and fluorescent DNA-binding dyes such as BrdU or Hoechst dye with FACS analysis), are all suitable assays to identify potential
modulators using a cell based system. Suitable cells for such cell based assays include both
primary cancer or tumor cells and cell lines, as described herein, e.g., A549 (lung), MCF7
(breast, p53 wild-type), H1299 (lung, p53 null), Hela (cervical), PC3 (prostate, p53 mutant),
MDA-MB-23 l (breast, p53 wild-type). Cancer cell lines can be p53 mutant, p53 null, or
express wild type p53. The N-cadherin protein may be
necessary. Transgenic animals generated by such methods find use as animal models of
the N-cadherin protein may be
necessary. Transgenic animals generated by such methods find use as animal models of

[0188] Cellular N-cadherin polypeptide levels can be determined by measuring the level of
protein or mRNA. The level of N-cadherin protein or proteins related to N-cadherin are
measured using immunoassays such as western blotting, ELISA and the like with an antibody
that selectively binds, respectively, to the N-cadherin polypeptide or a fragment thereof. For
measurement of mRNA, amplification, e.g., using PCR, LCR, or hybridization assays, e.g.,
northern hybridization, RNAs protection, dot blotting, are preferred. The level of protein or
mRNA is detected using directly or indirectly labeled detection agents, e.g., fluorescently or
radioactively labeled nucleic acids, radioactively or enzymatically labeled antibodies, and the
like, as described herein.

[0189] Alternatively, N-cadherin expression can be measured using a reporter gene system.
Such a system can be devised using an N-cadherin protein promoter operably linked to a
reporter gene such as chloramphenicol acetyltransferase, firefly luciferase, bacterial
luciferase, β-galactosidase and alkaline phosphatase. Furthermore, the protein of interest can
be used as an indirect reporter via attachment to a second reporter such as red or green
fluorescent protein (see, e.g., Mistili & Spector, Nature Biotechnology 15:961-964 (1997)).
The reporter construct is typically transfected into a cell. After treatment with a potential
modulator, the amount of reporter gene transcription, translation, or activity is measured
according to standard techniques known to those of skill in the art.

C. Animal models

[0190] Animal models of cellular proliferation also find use in screening for modulators of
cellular proliferation. Similarly, transgenic animal technology including gene knockout
technology, for example as a result of homologous recombination with an appropriate gene
targeting vector, or gene overexpression, will result in the absence or increased expression of
the N-cadherin protein. The same technology can also be applied to make knock-out cells.
When desired, tissue-specific expression or knockout of the N-cadherin protein may be
necessary. Transgenic animals generated by such methods find use as animal models of
cellular proliferation and are additionally useful in screening for modulators of cellular proliferation.

[0191] Knock-out cells and transgenic mice can be made by insertion of a marker gene or other heterologous gene into an endogenous N-cadherin gene site in the mouse genome via homologous recombination. Such mice can also be made by substituting an endogenous N-cadherin, respectively, with a mutated version of the N-cadherin gene, or by, respectively, mutating an endogenous N-cadherin, e.g., by exposure to carcinogens.

[0192] A DNA construct is introduced into the nuclei of embryonic stem cells. Cells containing the newly engineered genetic lesion are injected into a host mouse embryo, which is re-implanted into a recipient female. Some of these embryos develop into chimeric mice that possess germ cells partially derived from the mutant cell line. Therefore, by breeding the chimeric mice it is possible to obtain a new line of mice containing the introduced genetic lesion (see, e.g., Capecchi et al., Science 244:1288 (1989)). Chimeric targeted mice can be derived according to Hogan et al., Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor Laboratory (1988), Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, Robertson, ed., IRL Press, Washington, D.C., (1987), and Pinkert, Transgenic Animal Technology: A Laboratory Handbook, Academic Press (2003).

D. Exemplary assays

Soft agar growth or colony formation in suspension

[0193] Normal cells require a solid substrate to attach and grow. When the cells are transformed, they lose this phenotype and grow detached from the substrate. For example, transformed cells can grow in stirred suspension culture or suspended in semi-solid media, such as semi-solid or soft agar. The transformed cells, when transfected with tumor suppressor genes, regenerate normal phenotype and require a solid substrate to attach and grow.

[0194] Soft agar growth or colony formation in suspension assays can be used to identify N-cadherin modulators. Typically, transformed host cells (e.g., cells that grow on soft agar) are used in this assay. For example, RKO or HCT116 cell lines can be used. Techniques for soft agar growth or colony formation in suspension assays are described in Freshney, Culture of Animal Cells a Manual of Basic Technique, 3rd ed., Wiley-Liss, New York (1994), herein incorporated by reference. See also, the methods section of Garkavtsev et al. (1996), supra, herein incorporated by reference.
Contact inhibition and density limitation of growth

[0195] Normal cells typically grow in a flat and organized pattern in a petri dish until they touch other cells. When the cells touch one another, they are contact inhibited and stop growing. When cells are transformed, however, the cells are not contact inhibited and continue to grow to high densities in disorganized foci. Thus, the transformed cells grow to a higher saturation density than normal cells. This can be detected morphologically by the formation of a disoriented monolayer of cells or rounded cells in foci within the regular pattern of normal surrounding cells. Alternatively, labeling with $[^3]$H-thymidine at saturation density can be used to measure density limitation of growth. See Freshney (1994), supra. The transformed cells, when contacted with cellular proliferation modulators, regenerate a normal phenotype and become contact inhibited and would grow to a lower density.

[0196] Contact inhibition and density limitation of growth assays can be used to identify N-cadherin modulators which are capable of inhibiting abnormal proliferation and transformation in host cells. Typically, transformed host cells (e.g., cells that are not contact inhibited) are used in this assay. For example, RKO or HCT116 cell lines can be used. In this assay, labeling index with $[^3]$H-thymidine at saturation density is a preferred method of measuring density limitation of growth. Transformed host cells are contacted with a potential N-cadherin modulator and are grown for 24 hours at saturation density in non-limiting medium conditions. The percentage of cells labeling with $[^3]$H-thymidine is determined autoradiographically. See, Freshney (1994), supra. The host cells contacted with a N-cadherin modulator would give arise to a lower labeling index compared to control (e.g., transformed host cells transfected with a vector lacking an insert).

Growth factor or serum dependence

[0197] Growth factor or serum dependence can be used as an assay to identify N-cadherin modulators. Transformed cells have a lower serum dependence than their normal counterparts (see, e.g., Temin, J. Natl. Cancer Inst. 37:167-175 (1966); Eagle et al., J. Exp. Med. 131:836-879 (1970)); Freshney, supra. This is in part due to release of various growth factors by the transformed cells. When transformed cells are contacted with a N-cadherin modulator, the cells would reacquire serum dependence and would release growth factors at a lower level.
Tumor specific marker levels

[0198] Tumor cells release an increased amount of certain factors (hereinafter "tumor specific markers") than their normal counterparts. For example, plasminogen activator (PA) is released from human glioma at a higher level than from normal brain cells [see, e.g., Gullino, Angiogenesis, tumor vascularization, and potential interference with tumor growth. In Mihich (ed.): "Biological Responses in Cancer." New York, Academic Press, pp. 178-184 (1985)]. Similarly, tumor angiogenesis factor (TAF) is released at a higher level in tumor cells than their normal counterparts. See, e.g., Folkman, Angiogenesis and cancer, Sem Cancer Biol. (1992)).

[0199] Tumor specific markers can be assayed to identify N-cadherin modulators which decrease the level of release of these markers from host cells. Typically, transformed or tumorigenic host cells are used. Various techniques which measure the release of these factors are described in Freshney (1994), supra. Also, see, Unkless et al., J. Biol. Chem. 249:4295-4305 (1974); Strickland & Beers, J. Biol. Chem. 251:5694-5702 (1976); Whur et al., Br. J. Cancer 42:305-312 (1980); Gulino, Angiogenesis, tumor vascularization, and potential interference with tumor growth. In Mihich, E. (ed): "Biological Responses in Cancer." New York, Plenum (1985); Freshney Anticancer Res. 5:1 11-130 (1985).

Invasiveness into Matrigel

[0200] The degree of invasiveness into Matrigel or some other extracellular matrix constituent can be used as an assay to identify N-Cadherin modulators which are capable of inhibiting abnormal cell proliferation and tumor growth. Tumor cells exhibit a good correlation between malignancy and invasiveness of cells into Matrigel or some other extracellular matrix constituent. In this assay, tumorigenic cells are typically used as host cells. Therefore, N-Cadherin modulators can be identified by measuring changes in the level of invasiveness between the host cells before and after the introduction of potential modulators. If a compound modulates N-Cadherin, its expression in tumorigenic host cells would affect invasiveness.

[0201] Techniques described in Freshney (1994), supra, can be used. Briefly, the level of invasion of host cells can be measured by using filters coated with Matrigel or some other extracellular matrix constituent. Penetration into the gel, or through to the distal side of the filter, is rated as invasiveness, and rated histologically by number of cells and distance moved, or by prelabeling the cells with $^{125}$I and counting the radioactivity on the distal side of the filter or bottom of the dish. See, e.g., Freshney (1984), supra.
Tumor growth in vivo

[0202] Effects of N-cadherin modulators on cell growth can be tested in transgenic or immune-suppressed mice. Knock-out transgenic mice can be made, in which the endogenous N-cadherin gene is disrupted. Such knock-out mice can be used to study effects of N-cadherin, e.g., as a cancer model, as a means of assaying in vivo for compounds that modulate N-cadherin, and to test the effects of restoring a wild-type or mutant N-cadherin to a knock-out mouse.

[0203] Knock-out cells and transgenic mice can be made by insertion of a marker gene or other heterologous gene into the endogenous N-cadherin gene site in the mouse genome via homologous recombination. Such mice can also be made by substituting the endogenous N-cadherin with a mutated version of N-cadherin, or by mutating the endogenous N-cadherin, e.g., by exposure to carcinogens.

[0204] A DNA construct is introduced into the nuclei of embryonic stem cells. Cells containing the newly engineered genetic lesion are injected into a host mouse embryo, which is re-implanted into a recipient female. Some of these embryos develop into chimeric mice that possess germ cells partially derived from the mutant cell line. Therefore, by breeding the chimeric mice it is possible to obtain a new line of mice containing the introduced genetic lesion (see, e.g., Capecchi et al, Science 244:1288 (1989)). Chimeric targeted mice can be derived according to Hogan et al, Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor Laboratory (1988) and Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, Robertson, ed., IRL Press, Washington, D.C., (1987). These knock-out mice can be used as hosts to test the effects of various N-Cadherin modulators on cell growth.

[0205] Alternatively, various immune-suppressed or immune-deficient host animals can be used. For example, genetically athymic "nude" mouse (see, e.g., Giovanella et al, J. Natl. Cancer Inst. 52:921 (1974)), a SCID mouse, a thymectomized mouse, or an irradiated mouse (see, e.g., Bradley et al, Br. J. Cancer 38:263 (1978); Selby et al, Br. J. Cancer 41:52 (1980)) can be used as a host. Transplantable tumor cells (typically about $10^6$ cells) injected into isogenic hosts will produce invasive tumors in a high proportions of cases, while normal cells of similar origin will not. Hosts are treated with N-cadherin modulators, e.g., by injection. After a suitable length of time, preferably 4-8 weeks, tumor growth is measured (e.g., by volume or by its two largest dimensions) and compared to the control. Tumors that have statistically significant reduction (using, e.g., Student's T test) are said to have inhibited
growth. Using reduction of tumor size as an assay, N-cadherin modulators which are capable, e.g., of inhibiting abnormal cell proliferation can be identified.

VI. Screening Methods

[0206] The present invention also provides methods of identifying compounds that inhibit the binding of a N-cadherin protein, respectively, to a N-cadherin receptor, wherein said compounds find use in inhibiting the growth of and promoting the regression of a tumor that expresses N-cadherin protein, for example a urogenital cancer tumor, including a prostate or bladder cancer tumor.

[0207] Using the assays described herein, one can identify lead compounds that are suitable for further testing to identify those that are therapeutically effective modulating agents by screening a variety of compounds and mixtures of compounds for their ability to decrease or inhibit the binding of a N-cadherin protein to a N-cadherin receptor. Compounds of interest can be either synthetic or naturally occurring.

[0208] Screening assays can be carried out in vitro or in vivo. Typically, initial screening assays are carried out in vitro, and can be confirmed in vivo using cell based assays or animal models. For instance, proteins of the regenerating gene family are involved with cell proliferation. Therefore, compounds that inhibit the binding of a N-cadherin protein to a N-cadherin receptor can inhibit cell proliferation resulting from this binding interaction in comparison to cells unexposed to a test compound. Also, the binding of a N-cadherin protein to a N-cadherin receptor is involved with tissue injury responses, inflammation, and dysplasia. In animal models, compounds that inhibit the binding of a N-cadherin protein to its receptor can, for example, inhibit wound healing or the progression of dysplasia in comparison to an animal unexposed to a test compound. See, for example, Zhang, et al., World J Gastroenter (2003) 9:2635-41.

[0209] Usually a compound that inhibits the binding of N-cadherin to a N-cadherin receptor is synthetic. The screening methods are designed to screen large chemical libraries by automating the assay steps and providing compounds from any convenient source to assays, which are typically run in parallel (e.g., in microtiter formats on microtiter plates in robotic assays).

[0210] The invention provides in vitro assays for inhibiting N-cadherin binding to its receptor in a high throughput format. For each of the assay formats described, "no modulator" control reactions which do not include a modulator provide a background level of N-cadherin binding interaction to its receptor or receptors. In the high throughput assays of
the invention, it is possible to screen up to several thousand different modulators in a single
day. In particular, each well of a microtiter plate can be used to run a separate assay against a
selected potential modulator, or, if concentration or incubation time effects are to be
observed, every 5-10 wells can test a single modulator. Thus, a single standard microtiter
plate can assay about 100 (96) modulators. If 1536 well plates are used, then a single plate
can easily assay from about 100- about 1500 different compounds. It is possible to assay
many different plates per day; assay screens for up to about 6,000-20,000, and even up to
about 100,000-1,000,000 different compounds is possible using the integrated systems of the
invention. The steps of labeling, addition of reagents, fluid changes, and detection are
compatible with full automation, for instance using programmable robotic systems or
"integrated systems" commercially available, for example, through BioTX Automation,
Conroe, TX; Qiagen, Valencia, CA; Beckman Coulter, Fullerton, CA; and Caliper Life
Sciences, Hopkintin, MA.

[0211] Essentially any chemical compound can be tested as a potential inhibitor of N-
cadherin binding to its receptor for use in the methods of the invention. Most preferred are
generally compounds that can be dissolved in aqueous or organic (especially DMSO-based)
solutions are used. It will be appreciated that there are many suppliers of chemical
compounds, including Sigma (St. Louis, MO), Aldrich (St. Louis, MO), Sigma-Aldrich (St.
Louis, MO), Fluka Chemika-Biochemica Analytika (Buchs Switzerland), as well as providers
of small organic molecule and peptide libraries ready for screening, including Chembridge
Corp. (San Diego, CA), Discovery Partners International (San Diego, CA), Triad
Therapeutics (San Diego, CA), Nanosyn (Menlo Park, CA), Affymax (Palo Alto, CA),
ComGenex (South San Francisco, CA), and Tripos, Inc. (St. Louis, MO).

[0212] In one preferred embodiment, inhibitors of the N-cadherin receptor binding
interaction are identified by screening a combinatorial library containing a large number of
potential therapeutic compounds (potential modulator compounds). Such "combinatorial
chemical or peptide libraries" can be screened in one or more assays, as described herein, to
identify those library members (particular chemical species or subclasses) that display a
desired characteristic activity. The compounds thus identified can serve as conventional
"lead compounds" or can themselves be used as potential or actual therapeutics.

[0213] A combinatorial chemical library is a collection of diverse chemical compounds
generated by either chemical synthesis or biological synthesis, by combining a number of
chemical "building blocks" such as reagents. For example, a linear combinatorial chemical
library such as a polypeptide library is formed by combining a set of chemical building
blocks (amino acids) in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks.


EXAMPLES

[0216] The following examples are offered to illustrate, but not limit the claimed invention.
Example 1: NF kappa B reporter assay with TAL as negative control

[0217] As shown in FIGs. 2-3, NFKB reporter activity is increased in cells that express stable N-cadherin. This is true both in androgen replete and depleted media. Also shown is that NFKB activation correlates with the amount of N-cadherin expression, since LNCaP-Cl expresses more N-cadherin than C2 and C3.

Example 2

[0218] As shown in FIGs. 4-5, N-cadherin is expressed on the cell surface of LNCaP-Cl cells, and that these cells have both higher amounts of NFKB expression, but that NFKB is activated, as shown by the strong nuclear signal in C1 cells. Control cells also express NFKB, but it is localized primarily to the cytoplasm.

Example 3

[0219] FIGs. 6-7 show that NFKB activation by N-cadherin showing upregulation of the NFKB target genes TGF β, IL-6, IL-8, and bcl-2.

Example 4

[0220] As shown in FIG. 8, knockdown of N-cadherin causes reduction in NFKB target gene expression, such as IL-6 and IL-8, again showing that N-cadherin regulated NFKB

Example 5

[0221] FIG. 9 shows NFKB promoter activity before and after treatment with N-cadherin targeted antibodies. The data show that there is immediate upregulation of NFKB mediated by these antibodies, which is followed at 7 days by downregulation. These data show that the antibodies may work in part by altering NFKB downstream signal transduction.

Example 6

[0222] FIG. 10 shows the staining of N-cadherin with and without treatment with N-cadherin targeted antibodies. In control treated cells, most N-cadherin is seen intracellularly; while in the presence of antibody, N-cadherin expression is stabilized on the cell surface. These data suggest that stabilization of N-cadherin on the cell surface is another mechanism of action of N-cadherin antibodies. This likely results in alterations in beta-catenin signaling that are mediated through cleavage and internalization of N-cadherin by metallopeptidases such as ADAM 10, among others.
Example 7

[0223] As shown in FIG. 11, N-cadherin downregulation can cause reductions in activation of the PI3K/Akt signaling pathway.

Example 8

[0224] As shown in FIG. 12, the treatment of cells with N-cadherin targeted antibodies causes alterations (both up and down) of Akt phosphorylation or activation, which supports the notion that these antibodies work, all or in part, by altering PI3K-Akt signal transduction.

Example 9: FACS analysis showing screening of antibody clones targeting the first extracellular domain of the N-cadherin protein

[0225] FIG. 13 shows recognition of the protein on the surface of prostate cancer cells.

Example 10: FACS analysis showing screening of antibody clones targeting the fourth extracellular domain of the N-cadherin protein

[0226] FIG. 14 shows that clones recognize N-cadherin on the surface of prostate cancer cells.

Example 11: Subcloning of N-cadherin antibodies

[0227] FIG. 15 shows that purified monoclonal clones recognize the protein on the cell surface.

Example 12: *In vitro* invasion assay.

[0228] FIG. 16 shows that the monoclonal antibodies IF12, 1H7 and 2B3 generated by the inventors' group all inhibit the invasiveness of N-cadherin positive cancer cells. GC4 is a control.

Example 13: Antibodies against N-cadherin block tumor growth and metastasis of PC3 prostate cancer cells, but have no effect on N-cadherin null tumors.

[0229] PC3 cells were implanted subcutaneously and grown. As shown in FIG. 17b, when tumors reached palpable stage at day 15, antibodies or control were given twice weekly (200 micrograms) for two weeks. Both 1H7 and EC4 were able to slow growth of the tumors. They also completely blocked metastasis. 5/5 control mice had lymph node metastases, compared with 0/5 and 0/5 1H7 and EC4 treated mice, respectively. In contrast, the 1H7 and EC4 antibodies had no effect on the growth of N-cadherin null tumors (FIG. 17a). FIGs. 17c and 17d show clear inhibitory effects of N-cadherin antibodies on the growth of PC3 tumors,
either in treatment of large established tumors (treatment starting on Day 21) or in long term treatment (treatment lasting as long as 62 days).

Example 14: Tumors from in vivo experiment show affect of antibodies against N-cadherin

[0230] As shown in FIG. 18, the two control tumors are very red, consistent with profound angiogenesis. They are also deeply adherent to and invasive into the local flank musculature. In contrast, the 1H7 and EC4 treated mice have pale, clear tumors that do not invade into local muscle. The tumors peel easily off of the underlying tissues, which demonstrates inhibition of angiogenesis and blockade of local invasion by the anti-N-cadherin antibodies, consistent with the finding that these mice had no metastatic disease.

Example 15: Immunohistochemical staining

[0231] The inventors have carried out immunohistochemical staining of an androgen independent LAPC 9 prostate cancer. The result shows that N-cadherin is only expressed by a small subset of cells (FIG. 19a).

Example 16: Androgen dependent and independent LAPC-9 tumor growth

[0232] To determine whether N-cadherin can be a target for treatment and diagnosis even though N-cadherin is not overexpressed in the cells or only expressed in a subset of cells, the inventors have studied the tumor growth of androgen dependent and independent LAPC-9 tumors. The androgen dependent and independent LAPC-9 tumors were treated with control PBS or N-cadherin antibodies 1H7 and EC4, respectively. The results show that even though N-cadherin is expressed in only a small subset of the androgen independent cells, that treatment with antibody is sufficient to delay the growth and progression of the androgen independent tumors (FIG. 20). These results suggest that the N-cadherin population of cells is required for androgen independent tumor formation, and that blocking it is sufficient to delay tumor progression. These results are consistent with an interpretation that N-cadherin marks a population of androgen independent stem cells. Blocking growth of the stem cells is enough to block growth of the tumor. These results also show that antibodies may work on cells that express normal or even low levels of N-cadherin.

Example 17: Sorted and unsorted N-cadherin positive and negative tumor cell growth

[0233] In order to determine the effects of N-cadherin positive cells on tumor cell growth, N-cadherin positive and negative cells were sorted, yielding a population of cells that were 100% and 0% positive for N-cadherin, respectively. Cells were then injected into castrate
mice, and the N-cadherin positive cells formed tumors more quickly and efficiently than the negative population (FIG. 20), suggesting that N-cadherin positive cells are either have a growth advantage, or that they have stem cell characteristics and are more tumorigenic than the negative population. Unsorted cells grow similar to the N-cadherin positive cells.

Example 18: FACS analysis of tumors from N-cadherin sorted cells

[0234] FACS analysis of tumors that grew from purely N-cadherin positive and negative cells, vs the control unsorted population. Tumors from 100% N-cad positive cells are only 41.25% positive for N-cadherin (FIGs. 21 and 22), suggesting that these cells give rise to N-cadherin null cells. This is consistent with the hypothesis that N-cadherin positive cells are stem cells that can give rise to more differentiated, N-cadherin negative cells. Meanwhile, the N-cadherin negative population gives rise to tumors that are 9% N-cadherin positive (FIGs. 21 and 22), similar to the unsorted cells (FIGs. 21 and 22). This suggests that growth of these cells requires that a stem-like population acquire or upregulated N-cadherin in order to form androgen independent tumors. The delay in tumorigenicity is caused by the requirement for N-cadherin to give rise to androgen independent tumors.

Example 19: Growth of established LNCaP-Cl tumors are inhibited by N-cadherin antibodies

[0235] As shown in FIG. 23, N-Cadherin antibodies 1H7 and EC4 were administered to mice bearing LNCaP-Cl tumors from Day 45 to Day 56, and their inhibitory effects on tumor growth were apparent by Day 72.

Example 20: N-cadherin antibodies inhibit growth of LAPC-9 androgen independent tumors

[0236] As shown in FIG. 24a, N-Cadherin antibodies 1H7 and EC4 were administered to mice bearing established LAPC-9 androgen independent tumors (passage 7) at 10 mg/kg twice a week starting on Day 15. The inhibitory effect is apparent by Day 30. The inhibitory effects of 1H7 and EC4 on large established LAPC-9 androgen independent tumors are shown in FIG. 24b, where the animals did not receive antibody treatment until Day 17.

Example 21: Dose dependent growth inhibition by EC4 antibody

[0237] As shown in FIG. 25, growth of PC3 tumors in nude mice was inhibited by the EC4 antibody (administered from Day 13 to Day 27), with a more prominent inhibitory effect observed in experiments where a higher dose of the antibody was administered.
Example 22: Toxicity study

[0238] Because N-cadherin is widely expressed in a variety of tissues, its potential toxicity is a concern if anti-N-cadherin antibodies are to be used as therapeutic agents. The present inventors have found that the 1H7 and EC4 antibodies cross-react with murine N-cadherin but no in vivo toxicity has been observed in either long-term or dose escalation studies in mice.

Example 23: N-cadherin antibodies inhibit androgen independent progression of LAPC-9 androgen dependent tumors

[0239] As shown in FIGs. 26a and 26b, N-Cadherin antibodies 1H7 and EC4 effectively inhibited the androgen independent progression of LAPC-9 androgen dependent tumors in castrated mice. In the first study (FIG. 26a), the antibodies were administered from Day 0 to Day 31 and the animals were observed for 45 days. In the second study (FIG. 26b), the effect of long term antibody treatment was observed. 1H7 showed moderate delay in tumor progression, whereas EC4 showed a more prolonged effect in blocking tumor progression.

Example 24: N-cadherin positive cells have growth advantage in castrated mice

[0240] As shown in FIG. 27, N-cadherin positive LAPC-9 tumors showed growth advantage over N-cadherin negative LAPC-9 tumors in castrated SCID mice. On the other hand, FIG. 28 illustrates the inversed correlation between N-cadherin expression level and androgen receptor expression in LAPC-9 androgen independent cells: along with the successive passages, the LAPC-9 cells progressively gained androgen independence while their expression of N-cadherin increased and their expression of androgen receptor decreased.

Example 25

[0241] The data in Figure 29 show that even though N-cadherin is expressed in only a small subset of the androgen independent cells, that treatment with antibody is sufficient to delay to growth and progression of the androgen independent tumors (pink and yellow curves). These data suggest that the N-cadherin population of cells is required for androgen independent tumor formation, and that blocking it is sufficient to delay tumor progression. These data are consistent with an interpretation that N-cadherin marks a population of androgen independent stem cells. Blocking growth of the stem cells is enough to block growth of the tumor. These data also show that antibodies may work on cells that express normal or even low levels of N-cadherin.
As shown in Figure 30, cells were injected into castrate mice, and the N-cadherin positive cells formed tumors more quickly and efficiently than the negative population, suggesting that N-cadherin positive cells are either have a growth advantage, or that they have stem cell characteristics and are more tumorigenic than the negative population. Unsorted cells grow similar to the N-cadherin positive cells.

As shown in Figures 31 and 32, tumors from 100% N-cad positive cells are only 41.25% positive for N-cadherin, suggesting that these cells give rise to N-cadherin null cells. This is consistent with the hypothesis that N-cadherin positive cells are stem cells that can give rise to more differentiated, N-cadherin negative cells. Meanwhile, the N-cadherin negative population gives rise to tumors that are 9% N-cadherin positive, similar to the unsorted cells. This suggests that growth of these cells requires that a stem-like population acquire or upregulated N-cadherin in order to form androgen independent tumors. The delay in tumorigenicity is caused by the requirement for N-cadherin to give rise to androgen independent tumors.

Example 26: Identification of the epitope for the monoclonal antibody designated EC4

Subtractive MALDI-TOF (epitope extraction) was used to map the binding site of the monoclonal antibody EC4. An overlapping synthetic peptide library was created from the N-cadherin 2 sequence:

ENPYFAPNPKIIRQEEGLHAGTMLTFTTAQDPDRYMQQNIRYTKLSDPANWLKIDPVNGQIT TIAVLDRESPNVKNINIYATFLASDNGIPPMSTGTLQIYLDINDNAPQVLP

Eleven of the 12 peptides were used in epitope extraction experiments with purified EC4 mAb. Repeat experiments were performed at various temperatures and various peptide:EC4 mAb ratios. Consistent epitope extraction results were observed with peptide #6 (SDPANWLKIDPVNG) indicating that this is the EC4 mAb binding site.

The library consisted of 12 peptides, all 14 aa in length (except #12 which had 16 aa), with 5 aa overlap between peptides (see, Figure 35). Eleven peptides were synthesized with no issues. Upon receipt, all were analyzed by MALDI-TOF at Vital Probes (VPI) and determined to be of good quality for epitope extraction experiments. The peptide producer (Genscript Corp) attempted synthesis and purification of peptide #7 several times. On the last attempt, the peptide was able to be purified and was sent to VPI. However, upon further analysis at VPI using MALDI-TOF it was determined that this peptide was of low quality and could not be used for epitope extraction. Epitope extraction then proceeded using all other peptides.
Peptides were then combined into three groups, or slurries, based on their observed molecular weight. Peptides with molecular weights different enough from each other to be easily detected by MALDI-TOF were combined (data not shown). After combination, MALDI-TOF was performed to detect the peptides and validate the slurries. For all three slurries, individual peptides were detected (data not shown).

Epitope extraction was performed by incubating the EC4 mAb with each peptide slurry. In total, four combinations were tested for each slurry: (1) Peptide:EC4 ratio of 1:1, incubation at 4C, (2) Peptide:EC4 ratio of 1:10, incubation at 4C, (3) Peptide:EC4 ratio of 1:1, incubation at 37C, and (4) Peptide:EC4 ratio of 1:10, incubation at 37C. After incubation, each reaction was spotted onto a stainless steel MALDI-TOF plate with MALDI-TOF matrix and data collected. This process was performed on two separate occasions.

A peptide is scored as extracted if it is significantly reduced or missing in an experiment containing antibody as compared to one without antibody (negative control). For this project, peptide #6 (SDPANWLKIDPVNG) was consistently reduced or missing after exposure to antibody as compared to the peptide-only control. Thus, based on these data, peptide #6 contains most if not all of the antibody binding epitope. Since peptide #7 was not able to be used in this project it can not be ruled out that it too contains some additional amino acids within a linear epitope to which the EC4 antibody binds.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications, including GenBank Accession Numbers, cited herein are hereby incorporated by reference in their entirety for all purposes.
WHAT IS CLAIMED IS:

1. An N-cadherin antibody that binds to the N-cadherin epitope SDPANWLKIDPVNG (SEQ ID NO: 10) or any portion thereof.

2. The antibody of claim 1, wherein the antibody competes with EC4 for binding to N-cadherin.

3. The antibody of claim 1, wherein the antibody binds to the N-cadherin amino acid sequence SDPANWLKIDPVNGQITTIAVLD (SEQ ID NO: 17).

4. The antibody of claim 1, wherein the antibody binds to the N-cadherin amino acid sequence QQNIRYTKLSDPANWLKIDPVNGQITTIAVLD (SEQ ID NO: 18).

5. The antibody of any of claims 1-4, wherein the antibody is a monoclonal antibody, an scFv fragment, a diabody, or a minibody.

6. The antibody of any of claims 1-5, wherein the antibody is a humanized antibody.

7. The antibody of any of claims 1-6, wherein the antibody is linked to a detectable label.

8. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and the antibody of any of claims 1-7.

9. The pharmaceutical composition of claim 8, wherein the antibody is a monoclonal antibody.

10. The pharmaceutical composition of claim 8 or 9, wherein the antibody is a humanized antibody.

11. A method of detecting a cell expressing N-cadherin in a biological sample, the method comprising contacting the biological sample with an antibody of any of claims 1-7, and detecting the presence of the antibody.

12. A method of treating, preventing, or ameliorating a disease associated with the overexpression of N-cadherin, the method comprising administering the antibody of any of claims 1-7 to an individual in need thereof.
13. The method of claim 12, wherein the disease is prostate cancer or bladder cancer.

14. The method of claim 12 or 13, wherein proliferation of a cancer cell which expresses or overexpresses N-cadherin in the individual is inhibited following the administering step.

15. A method for identifying a modulator of N-cadherin, the method comprising:
   (a) contacting a test agent to a polypeptide comprising the amino acid sequence SDPANWLKIDPVNG; and
   (b) selecting an agent that binds to the polypeptide, wherein binding of the test agent to the polypeptide indicates that the test agent is a modulator of N-cadherin activity.

16. The method of claim 15, wherein the selecting step comprises measuring the ability of the test agent to compete with EC4 for binding to the polypeptide.

17. The method of claim 15, wherein the polypeptide is no longer than 35 amino acids long.

18. A polypeptide no more than 35 amino acids in length and comprising the amino acid sequence SDPANWLKIDPVNG.

19. The polypeptide of claim 18, consisting of at least 24 contiguous amino acids of the sequence QQNIRYTKLSDPANWLKIDPVNGQITTTIAVLD.

20. The polypeptide of claim 18 having the amino acid sequence SDPANWLKIDPVNG.


22. A nucleic acid encoding the polypeptide of any of claims 18-20.

23. A vector comprising the nucleic acid of claim 22.

24. A method of treating a cancer which expresses or overexpresses N-cadherin in a subject in need thereof by administering the vaccine of claim 21 to the subject.
25. The method of claim 24, wherein the cancer is prostate cancer or bladder cancer.

26. A method of treating a cancer which expresses or overexpresses N-cadherin in a subject in need thereof by administering the vector of claim 23 to the subject.

27. The method of claim 26, wherein the cancer is prostate cancer or bladder cancer.
Model of N-cadherin signaling in prostate cancer

[Diagram with labels: N-Cad, PDGF, N-Cad, Beta-catenin, N-cad E, N-cad ID, Cleavage by ADAM-10, IKK, NF-kB, AR, AKT, To nucleus, Target genes]

Target gene Expression: IL-8, 6, bcl-2, TGFβ

FIGURE 1
N-cadherin Activates NF-κB

Normal serum

NF kappa B reporter assay: TAL is negative control

FIGURE 2
N-cadherin Activates NF-κB

Normal serum

DCC serum
Androgen depleted

FIGURE 3
N-cadherin is expressed on cell surface of C1 cells

FIGURE 4
NF-κB localizes to nucleus in N-Cadherin positive cells (C1)

FIGURE 5
N-Cadherin Expression Results in Induction of IL-6, IL-8, TGF β 2, and bcl-2

**FIGURE 6**
Correlation of Induced Genes with N-Cadherin Level

<table>
<thead>
<tr>
<th></th>
<th>LNCaP-FGC</th>
<th>LNCaP:NCad1</th>
<th>LNCaP:NCad2</th>
<th>LNCaP:NCad3</th>
<th>LNCaP CL-M</th>
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<tbody>
<tr>
<td>N-cadherin</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>E-cadherin</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>AR</td>
<td></td>
<td></td>
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<tr>
<td>TGFβ1</td>
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<tr>
<td>IL-6</td>
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<td>IL-8</td>
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</tr>
<tr>
<td>GAPDH</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

FIGURE 7
N-Cadherin Knockdown Leads to Downregulation of IL-6 and IL-8

LNCap NCad C1

<table>
<thead>
<tr>
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<th>48hr post trans</th>
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<tr>
<td>Ncad</td>
<td>NC si N-Cad si</td>
<td>NC si N-Cad si</td>
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<tr>
<td></td>
<td>100 65</td>
<td>100 95</td>
</tr>
<tr>
<td>Ecad</td>
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<tr>
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<td>IL-6</td>
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<tr>
<td>IL-8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
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<td></td>
</tr>
</tbody>
</table>

FIGURE 8
NFκB activity after N-Cadherin antibodies treatment

NFκB activity after 7 days treatment of 1H7, reduced slightly. This is in agreement with the western blot where pAkt and Ncad expression was reduced.

FIGURE 9
Ncad in PC3 cells: 48hr antibody incubation
(with fibronectin)

(From previous)

1xPBS

1H7

EC4

FIGURE 10
**N-Cadherin Knockdown Leads to Downregulation Of Activated Akt**

**FIGURE 11**

N and N si = N-cadherin specific siRNA; NC si and C are controls
N-cadherin specific antibodies activate, then downregulate Akt activation

<table>
<thead>
<tr>
<th></th>
<th>1H7</th>
<th></th>
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<th>EC4</th>
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<tr>
<td></td>
<td>PBS</td>
<td>6hr</td>
<td>24hr</td>
<td>PBS</td>
<td>6hr</td>
<td>24hr</td>
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<td>Ncad</td>
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</tr>
<tr>
<td>pAkt</td>
<td></td>
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<tr>
<td>Akt</td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

FIGURE 12
N-cadherin Antibodies Have No Effect on N-cadherin Null Tumors (negative control study)
N-Cadherin Antibodies Inhibit Growth of Established PC3 Tumors

Treatment started when tumor palpable on Day 13; 10 mg/kg twice weekly for two weeks

FIGURE 17b
N-cadherin Antibodies Inhibit Growth of Large Established PC3 Tumors

Treatment started when tumor 100\(\text{mm}^3\) on Day 21: 10 mg/kg twice weekly for two weeks

FIGURE 17c
Long-term Treatment with N-cadherin Antibodies Inhibit Growth of PC3 Tumors

Ncad antibody experiment: PC3 EC4 long-term injection in nudes

FIGURE 17d
N-cadherin Antibodies Block Local Invasion and Angiogenesis of PC3 tumors

FIGURE 18
a.

b.

LAPC9AD to Al transition: 1xPBS vs 1H7 vs EC4

FIGURE 19
Tumor Growth Curve of LAPC9AI sort

FIGURE 20
FIGURE 21
FIGURE 22
Growth of established LNCaP-C1 tumors are inhibited by N-cadherin antibodies

LNCaP-Ncleone1 Ncad antibody established tumor exp:
1xPBS vs 1H7 vs EC4 (adjusted)

FIGURE 23
N-cadherin antibodies inhibit growth of established LAPC-9 androgen independent tumors (passage 7)

LAPC9Al passage7: 1xPBS vs 1H7 vs EC4

10 mg/kg 2X/week for two weeks, start day 15

FIGURE 24a
N-cadherin antibodies inhibit growth of large established LAPC-9 androgen independent tumors.

EMT-x1 antibody experiment: LAPC9Al p6 1H7 vs EC4 late injection

<table>
<thead>
<tr>
<th>Tumor volume (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2500</td>
</tr>
<tr>
<td>2000</td>
</tr>
<tr>
<td>1500</td>
</tr>
<tr>
<td>1000</td>
</tr>
<tr>
<td>500</td>
</tr>
</tbody>
</table>

Days: 0, 3, 6, 8, 10, 13, 15, 17, 20, 23, 25, 28, 30

Begin Tx: approx. 100mm³
End Tx: approx. 100mm³
Dose Dependent Growth Inhibition by EC4

EC4 dosage experiment: PC3 in nude mice

Tumor Volume (mm³)

Days

FIGURE 25
N-cadherin antibodies inhibit androgen independent progression of LAPC-9 androgen dependent tumors

LAPC9AD to AI transition: 1xPBS vs 1H7 vs EC4

LAPC-9 AD in intact mice/LAPC-9 AD in castrate mice with PBS/1H7/EC4

FIGURE 26a
N-cadherin antibodies inhibit androgen independent progression of LAPC-9 androgen dependent tumors

FIGURE 26b
N-cadherin Sorted Cells Have Growth Advantage in Castrate Mice

FIGURE 27

LAPC9AI sorted in castrated SCID

(Units:肿瘤体积 (mm)³)

days

N(+)sort
N(-)sort
Nunsort
N-Cadherin Expression Increases and AR Decreases with Successive Passages in LAPC9AI
FIGURE 29
Tumor Growth Curve of LAPC9AI sort

![Graph showing tumor growth over time for LAPC9AI sorted in castrated SCIDs]

**FIGURE 30**
FACs results on processed N-cad sorted tumors

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Marker</th>
<th>% of Gated Cells</th>
<th>Ave%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ncad(+)-sort</td>
<td>FL4-H</td>
<td>None</td>
<td>41.25%</td>
</tr>
<tr>
<td>Ncad(-)-sort</td>
<td>FL4-H</td>
<td>None</td>
<td>8.58%</td>
</tr>
<tr>
<td>Ncad unsort</td>
<td>FL4-H</td>
<td>None</td>
<td>9.95%</td>
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</table>

FIGURE 31
FIGURE 32
FIGURE 33
Human N-Cadherin Protein Sequence Information (SEQ ID NO:1)

1 mcriagalt lpllaallga svesageial cktgfpvedy savlklvdhe gqpllnvkfs
61 ncnkrrkvy esspadfkn dgedmyavrf sfplssheb fliyaqdkt qekwgvavkl
121 slkgpttees vkesaeveel vfprfqskshe ghllrgqkrd wippinilpen srqpfqgclv
181 risrdrknln slrystvtgpg adqgqfpgfifi inpsqglsyv tkpldrqegia rflrhavd
241 ingnqvenpi divindvm dhrephfihq wngtqepgsk pgyvmtvta idaddpnain
301 gmirryrvsq apstgspmnf tinmetgdil tvagldre vqgyttliiga tdmeqmpytg
361 lnssntatv vtdvndhppe ftamtyfev gev penrvdirda nltvcdkdpq htpawmvyr
421 isggdpbrvf aigtdpnsnad givtvpqpid fetnrvmvl vaenqvpila kgqhpqapvst
481 atsvsvbtvdv nepypapnp kqirgegelh agtmitftta iqdpdvcqnpn qtryrdlhep
541 nwllkidpyn gqittavdlr espnvknnny natfasdng ipmpssgtglq yqylldindn
601 apqvpqeaa tcectdpnsi nitalydid pgagpfadfl plapvtirkn wtitrinpjdf
661 aqinllkkfl eaqgcvyvpi itdegqppks nisllrvkqv qcdngcdont vdrayvaggl
721 tgailailce iiilililivm fwwvnrkrdk errgakgllid pedtvrdhil kydeeggge
781 dgdydlsqal gpqtvdpnaip kpvytjrrme rpihaegqyp vrsaahpgpd igdfingik
841 aanddptapp ydshllvdyeq geseltgls sllnssssg qdydylndwp prkkladmy
901 ggdd

ORIGIN

1 atgtgcggaaga tagggcgagg gctgtcggcagc ctgcggctgc gttggcccttc gcctggagccg
61 ctctgttagagg cctttcgttgata aaccgtcctag tgcaagactg tattctctga atagttcttac
121 agttgctcagc atgtcgcatag ttctcggtaag gatccgctct aatctctctctg gatggtagttgac
181 aaagctcaaat ctgaaagagca agtacgccag tgcaccgctg aagagagagag aacacccacta
241 gagttgcttgatg atggcctggta atgtaggctag tgcagctgatt gcatactctctc tccggaggtct
301 ttctcggagca cctttctcagt aatctctctctg gatggtagttgac
361 gcctgagcttggt cctggaggtta atgtaggctag tgcagctgatt gcatactctctc tccggaggtct
421 gcctgagcttggt cctggaggtta atgtaggctag tgcagctgatt gcatactctctc tccggaggtct
481 gcctgagcttggt cctggaggtta atgtaggctag tgcagctgatt gcatactctctc tccggaggtct
541 gcctgagcttggt cctggaggtta atgtaggctag tgcagctgatt gcatactctctc tccggaggtct
601 gcctgagcttggt cctggaggtta atgtaggctag tgcagctgatt gcatactctctc tccggaggtct
661 gcctgagcttggt cctggaggtta atgtaggctag tgcagctgatt gcatactctctc tccggaggtct
721 gcctgagcttggt cctggaggtta atgtaggctag tgcagctgatt gcatactctctc tccggaggtct
781 gcctgagcttggt cctggaggtta atgtaggctag tgcagctgatt gcatactctctc tccggaggtct
841 gcctgagcttggt cctggaggtta atgtaggctag tgcagctgatt gcatactctctc tccggaggtct
901 gcctgagcttggt cctggaggtta atgtaggctag tgcagctgatt gcatactctctc tccggaggtct
FIGURE 33 (Cont’d)

2041 atcagcagtt cggtagatt ccctggaaat ccctagctcag ctccagttag gcgcgttagttgc
cagtctgtac ccgacccgggc ctcgctcag attggtggtgct gggtttgtgccc
2161 accgttgcct ccttcgctgt cctgctgtcag cttgcctctct cgtcgtggtgatg
ttggtgtagt ggtgagaaaag cggtagctgaga gctgctgccag ccacacactact taattgat
2281 ccgagagatg atgtaaggag taattttttaaatagatgtg aagaaagttgg aggagagagaa
2341 gaccgcagcact gccgtgtgag cccagctcag ccgctggtac ccgctggtgc ctgtgcaggtc
cacgcctca ctagctctcc cttggctgtac ccagctcagcc ccagtcatccg
cggcagcact gcggccctag ccggcagcact gcggccctag ccggcagcact gcggccctag
cggcagcact gcggccctag ccggcagcact gcggccctag ccggcagcact gcggccctag
2521 gcggctgaca atgcgcctccag ctggccctcctgcttggtgttc tgcgtgatgag
2581 gcgcctgctg agcagctgctg gcgcctgctg gcgcctgctg gcgcctgctg gcgcctgctg
2641 cagactctgat agactctgat cagactctgat cagactctgat cagactctgat cagactctgat
2701 ggtgagggtg atgactgta

translation = "MCRIGA4LRTPLLA4LQQSVEASGGALACKTGFIPEDVVSAVL
SKDHEGQPLLNVFSNCNG2KQRVQYESSEP AKFVDEDMGYAVRSPFSLSEHAKFL
IYAQDKETQ8E8QVAVKLS4L48PKTTEESVKELESAEVEEIVFPRQFSKSHSLGQKRDW
VIPPPINPNSRGPFPQELWRISRSDKNLSLYSRTVGAPQGADDQPTGPFGIFIPIN8S0QL
SVTKFLDREQIARFHLRAH1VINGNQVENP4DIVINVIDMNDRPEFHLQVWN6TPV
EGSKPGTYVMTVTAIDADDPNALN4MLRLRVQAPISTSPSPNMFT1NETGY1ITVA
GLDE4KVQYTLI1QATDMGPNPT4GLSN4TATAVITDVTVDWDNPEFTAMTFYGBPE
NRRVI4VANLTVTDKQ8FPAWMVSNISGGDTGPRFAIQ8TDPNSN4GLVT0VSDKID
FETNRMFLVTVSAE4NVQVPLAK4IHQPPOQSTATSVTVTDVNEQPYPFNKII1QREBG
LHAGTMLOITFTAQDPDRYMQNIRYTKLS4PDW4L4KIDPVNQINTI1ADRESFNVK
NNYN4ATIASD4NGIPPMGSTGTLQIYLLIDINND4QVLPQAEFT1CTDPDNS4NITA
LDYDIPNAPGFFADPLSVP4T1KRNWTTITLNGDFAQLNLKIKFLEAGIYE1VI1IT
DSGPSNPSNISILR6V6QCCDSNGDCTDVDRIV4AGLGTGAIAILLCSIILLILVLM
FVWVKRDRD4KQAKQILIDFTEPDDVRDNILKYEDEGGGEEQDYDLSQLQPPPDTEVPD
AIKPVGIRMDER1PHAEPQYPVRSAPAPHPGDIDQFINEGLKAADNDTAPPYD4L4V
FDYE9GSGTGLS4SLN4SSSSG44EQO4DY1ND84GPRF4KLADMY4G4DD"
FIGURE 34

N-Cadherin variant protein (SEQ ID NO:3) and mRNA (SEQ ID NO:4) sequences and antibody binding information

LOCUS NM_001792  4122 bp  mRNA  linear
DEFINITION Homo sapiens cadherin 2, type 1, N-cadherin (neuronal) (CDH2), mRNA.

CDS  206..2926

SEQ ID NO:3

MCRIAGALRTLPLLALLQQSVEASGEIALCKTGFPEDVSYAVLSKDVHEGQPL
LNVKFSNCNGKRKVEQAYSEEPADKVFEDDMJVAYVRSFPLSSEHAKFLIYAQDK
ETEKEWQVAVKLSKPLTLEESVKESAEVEIVFPRQFSKHSHGLQROQKRDWViP
PINLPENSRGPQPELVIRSDRNKLNRSVTGPQADQPPTGIFINIPISGQLSVT
KPLDREQIARFHLRAHAVINGNQVENPIDIVINVIMNDRPFLHQQVWNGTYP
EGSKPGTYVMTVTADDPNALNGMLRVRIVSQAQPSTSPNMTITNETGDIIT
AAGLDREKVQVYTLIQTATMEGNTPYGLSNTATAVITVDVNDPPEFTAMTF
YGEPENRDIVIANLTVDKDPQHTPAWNAVYRISGĐPTGRFAIQTDPSNĐG
LVTVVKPIDFETNMFVLTVAENQVPLAKGIQHPPQSTATSVTDVTDVNENPYF
APNPKIIRQEEGLHAGTMLTTFTAQDPRYMQQNIYTKLSDPANWLKIDPVNG
QITTTAVLDRESPNVKNIIYNATFLASNPGIPMPGTSQTLQYLLIDNDAPQVLPQ
EAETCETPDPSNITALDYDPNPAGPFAFDLSPVTIKRNWTITRLNGDFAPQLN
LIKIFLEAGIYEVPIIIITDGSNPNKSNISLRVKVCQCDSNGCDTVDRIVGAGLGT
GAIIAILLCIIILILILVMFVMWMKRPDDRKEQAKQLIDPDEDVRNDLKYDEEGG
GEEDOQYDLSQLQQPDTVEPAIPIKVQGIRRMDRPIHAEPQYPVRTAAPHPGDIG
DFINEGLKAADNPATPAPYDSSLVFDFEGSGSTAGSLLSLSSSSGEQQDYDYLNDW
WGPRFKKLADMYGGGDD

Binding sites of antibodies: Ncad 1H7, 2B3, 1F12 - 21-425aa ;

SEQ ID NO:4

1 ttgtcatca gctcgtcctc cattgccccgg gacgagagag cagcgaagaac ggggtggtggg
61 aggaggaggg aaggaggaggg ggtggaact gctgtagagc ctttctcgc ggccgtgttg
121 ggtgtgcgccg ttgctctctc tctecgcgcg cgcgccggcg gcggcgcgcccgc gccggcgtcttg
181 ctctccccg ccctctccg ctctccatgg gcggtagccgg gcagctcgcgg gcagccctgctgtg
241 gcgctgttg gcgggctcgc gtcaggtggc tggagagaggt tggagtgaaac tggaggtattg
301 caagactgaa ttcctctgaag atgtttcag tgcagcttca tccaaggttgc gcatgaggg

Nead 1H7, 2B3, 1F12 - 266-1482
FIGURE 34 (Cont’d)

361 acagcctttc ctcaatgtga agttaagcaa ctgcaatgga aaaaagaag tacaatatga
421 gacgagttaac cctgcaagatt ttaaggttta gaagaatggc atggtgtat ccggtgagaag
481 ctctcacttc tctctgacag atgcgaatgt cacctatata gcacaagaca aagagaccca
541 ggaaagttgg caaagtcgag tcataatagc cctgaagccaa acctttactcg aggagtcatgt
601 gaagggactca gcagagatgg aagaataagtt gttctcaaga caattcactga agcagactgg
661 ccacctcaca aggcaagaaga gagaactggg ctcgccctca atcactactgc cagaaactc
721 caggggaacct tttctcaag aagctgtcgag gataaggtctc gatagagata aaaaactttc

781 actgcaagtac agttaacttg gcacaagaggc tcacagacct ccaactgtgtacttccttat instead of "a"

841 caacccacact tcgggtcagc tgctggtcag cacccagcct gatcggcagg agatatgcagc
901 gttctcatttg aaggccacag tcagatgat gtaattgacat caatgggaat caagtggaga accccacattga
961 cattcgtatc aatgtatttg acatgaatag caacagacct gatgttatgc accaggttttg

1021 gaatgggaca gttctgtgagg gataaagcct ggaacattat gttgatgcacg tatacacatt
1081 tgaatgctgac gataccaatgt ccctaatggg gagttgtgagc tacaagactcg tgctctaggc
1141 tcaagacacc ccttcacacca acatttttag tcataaacat gagaactgtgtg acatctcactc
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1321 gacgagatgc aatgacactc ctccagagtt tactgcaatgg acgttttatg gtgaagtttgc
1381 tgagaacaggg gtagacatca tagtagctaa tctaaactgtg accgataagg atcaacccca
1441 tacacacgcct tgtgaccagc tgtacagatat cagttgcggga gatcctactgt gcggttgcgc
1501 catcctagac ccaccacaac gcaagacaggg gttagteacce gttggtcataac caatcagactt
1561 tgaacacatat agatgttttt gccttaactgt tgcagcagga caggtgatgc cattgcaacca
1621 ggggaattcag caccgcccctt acgtcaactgc aaccgtgtct gtacagatg taagcgttaaa
1681 tgaacacacctattttgccc ccaataactaat gatcattgcg caagaagaag ggctctctatgc
1741 gggttacactc ttgcaacaact tcaactgctca gacccagat gatatatgc agaacaatat
FIGURE 34 (Cont’d)

1801 tagatacact aaattatcgt atctctgccca tiggctaaaat atagatcctg tgaatggaca
1861 aataactaca attgctgttt tggacgcaga atccaccaat gtgaaacaag atatatataa
1921 tgctaccttc ctgctttctg acaatggaat tctctctatg agtggaacag gaaegctgca
1981 gatctattta ctgtatatta atgacactgc ccctcaagtg ttacctcaag agggcagagac
2041 tggcgaacact ccagacccca atcaatattc attacacgca cttgattatg acattgtacct
2101 aatgtctgga ccatttgcct ttagcttctcc tttacttcca gtgcacttata agagaaaaatg
2161 gaccatacact cggcttaata cggagtttgc tcgactttaat tttaagatataa aatctctttga
2221 agctggtact tatgaagtct ccaatcaatt gcagattttg ggatacctct cccaaatcaca
2281 tattttcccc ctggctctga aagggggcctt gttgctactcc aacgggggac gccacagatgt
2341 ggagacgggatt cggctgctcc gggctggccac cgggtgctcctt attgcttctatc tctggtcatt
2401 cattatccttg cttatccttg tctgtgatgg tttgtgtatttg atgaaagcc gccgaaaaga
2461 acggcgggcc aaacacacttt taaaataagc cggagatatg ttaagagata atatatataaa
2521 atatgtgtgaa caggttgagag gagaagaaga ccaagactat gacttgagcc aagctgacgca
2581 gcctgacact gcggagccctg atgctcacca gcctgctggga atccgagaaa tggatggaag
2641 acccatccac gcctgagccccc agatcctcgg cgcatctgca gcgcacacce cttgagacat
2701 tggggacactt attataggg gcctttaaagc gcctgacata cacccccacag cttccacacta
2761 tgaactccttg ttagttttgc actataggaag cagttgctcct aagctggtgccttcttgagctc
2821 ccttaatccct ctaaagttgtg tgggtgagcca gaagatagtct tacctgaagct aagctgagggccc
2881 agcgttcaag aaactgtgctg acagttagg tggaggtgtg gactgacatt caggtgtggcaac
2941 ttgggtttttg gacaagataa aacaatttca actgatatcc ccacaaaaaca ttcagaagct
3001 aggcctttaac cttgtagct actagccacag tgcctgtctgg aagctttttgc aatggtgtgca
3061 aaccaatattg gcctcagaggg gaatatcag tatacatactg ttggggaatg aacaggtgagct
3121 cagttacact tgaatatttac agttcagaaaaa cacttgggatt ttactgactt ttttgtacct
3181 ttctcagatt ggaataggtt tctgttttaa gcggcttaatg gcagctgattt cttgaacgat
FIGURE 34 (Cont’d)

3241 aagttaaaga caaataattt tgttgttgga gcagtaagtt aaaccatgat atgcttcac
3301 acgcttttgt tacattgcat tgtcctttat taaaatacaca aattaaacaa acaaaaaaac
3361 taatggagcg attttttat ctggggggat gagaccaatga gattggaaaa tggacattac
3421 tttgatgtttt agacatgttga tgttttttttttttca actcttaaa aactctcag
3481 ctgggtgcaaa ataaagggagtt tttaacttac ccacatgtgat agcaaaaattg aattttttca
3541 taaactaggaa ttgtaagcag atttttgtcgt taatcctcatgt acactttttttt atttttttat
3601 tttccacctc ccgtgtaaaa atagtatgtg tacataatgt tttatgggca tagctctatgg
3661 aagaagtgcg aacactcaga acatgtgtat gtattatttg gactatggtc tcaaggttttt
3721 tgcatgttta tatactttctgt tatggataaa gtatatttacca aacagtgcac tttgatcaca
3781 ttggtagct gtggtagaa tactcaatg ttaatttttt taatttttttt atttttttttatt
3841 ttctttttgg tgttggaggg gagaagagt cttagcaca atggttttaca taattttgatc
3901 caaaaaaaaaa aaaaaagaaa aaaaagaaaag ggggtgcccgr acactgttgg acactaacg
3961 tgtgtggtgt taataataaaa aatggaaaaa aaaaaagctt ttaaactggga gagaactctg
4021 aacaagcagt gtcctctcgtac tttgatcaca gaatataaat gatacacttc tgacccgac
4081 gtctgataa aatgctaat atttggaaaa aaaaaaaa aa

Note the mutation at 808. When sequenced, it's a "g" instead of an "a" as published, codes for the same aa. The amino acids are highlighted in the attachment.
# Peptide Summary Information

*No information generated for peptide #7 due to impurity of product*

<table>
<thead>
<tr>
<th>Peptide Number</th>
<th>Sequence</th>
<th>Expected MW</th>
<th>Observed MW</th>
<th>Slurry ID</th>
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<td>Peptide #1</td>
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<td>1647.90 / 1669.28(Na⁺)</td>
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<td>1526.35</td>
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**FIGURE 35**